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Characteristic features of the microbiology of the Webster and Tama silt loams

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CHARACTERISTIC FEATURES OF THE MICROBIOLOGY OF THE WEBSTER AND TAMA SILT LOAMS

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INTRODUCTION

Since the recognition of the existence of a microbiological population in the soil and the demonstration that the activities of the microorganisms concerned have a great influence on soil fertility, attempts have constantly been made to construct some picture of the distribution of the soil population and to understand the relationships between the various groups of organisms composing it. The vast numbers of organisms apparently present, and the complexity of types, have made this no easy problem. Comparisons on a numerical basis have not been found to be particularly helpful, since the results obtained seem to be so greatly affected by the procedure adopted and distinction between active and resting forms is difficult. Attempts to obtain an index of the microbiological condition of the soil by the determination of those biochemical changes that the population could bring about have in most cases been recognized as being artificial. The well-known plate and dilution methods have given interesting and valuable data on the types of organisms present and their individual potentialities but have added little to the study of the population as a whole. However, increasing success is following the more straightforward approach of direct examination now that some of the many serious technical difficulties have been overcome.

The newer procedures are by no means perfect, and do not yet a full and complete picture of the various types in the soil microbial population. They are satisfactory in that the conditions involved in most cases are not highly artificial; they are unsatisfactory in that there is nothing that can be quantitatively expressed as yet. Comparisons of different soils, therefore, have to be made on the basis of the organisms observed, their

associations, and the frequency of their occurrence.

The work describes an attempt to follow by direct examination in place of cultural methods the characteristic features of the microbiology of two typical Iowa soils.

HISTORICAL

It has been a criticism of biologists that frequently they are apt to ignore the study of populations and to concentrate on the behavior of individuals which taken alone and out of their natural environment may not be typical of the population as a whole. This criticism may well be applied to those who have studied soil microorganic populations, methods for the examination of which can be divided into direct microscopic observations and pure cultural studies. The former should provide a picture of the population in a condition more or less natural according to the procedure which it is necessary to follow, while the latter cannot do more than indicate the types of organisms present and their individual potentialities. Cultural methods are, of course, selective in that the organisms appearing are only those which are able to grow on the medium used. Partly because soil microbiology has developed from the foundations of medical bacteriology and partly because satisfactory direct methods of examination have not proved easy to devise, pure cultural methods have been widely and almost exclusively used, although it is fair to say the limitations thereof have been pointed out by numerous workers.

Nevertheless the chief methods commonly in use for soil microbiological investigations are the so-called plate and dilution methods. For the laboratory teacher the plate method is one of the simplest methods, although indirect, to demonstrate to beginners that the soil contains life and at the same time to present some idea of the numbers of organisms present. For the research man it is a method which may give many data concerning the individual groups within the soil or soils under investigation but as mentioned before it is limited in its ability to yield complete information.

Inasmuch as the culture methods preceded the direct methods and have been so widely used these will be taken up first. It will not be necessary to give in detail the plating procedure for soil investigations as the steps can be found in bacteriological laboratory manuals. As the dilution method is the same as the plate procedure except that one cubic centimeter of several of the final dilutions is plated on a number of special media adapted to the growth of particular groups of organisms only a brief description of the plate procedure will be given. A small amount of the soil under investigation or if information as to numbers is required, a weighed amount, is added to sterile water and then shaken for a sufficiently long time to disperse the soil and to break up the colonies of microorganisms. From this a series of dilutions is made so that one cubic centimeter of the final dilution when plated will give from 40 to 200 colonies on either agar or gelatin plates. One cubic centimeter of the final dilution is added to petri dishes and melted agar or gelatin, sufficiently cooled, is poured into the plates which are then incubated. This method may be used for both bacteriological and mycological studies of soils but a medium which will support fungus growth and suppress the development of bacterial colonies is desirable in the case of fungous investigations.

The obvious shortcomings of these methods are that neither closely simulates the natural environments of the microorganisms, and that both are physiologically selective according to the different nutrients added to the agar. This defect was recognized by Conn (5) in 1917. In his discussion of the methods then in use for counting microorganisms he stated, "the only satisfactory method yet devised for determining the numbers of bacteria in soil is by means of poured plates. It has long been recognized that this

method does not give a complete count. There are bacteria in soil that do not grow in our ordinary culture media, and as this method gives a count of those organisms alone that are able to produce macroscopic colonies in agar or gelatin, those that do not grow are overlooked".

Conn (10) made the first suggestion concerning the study of soil microorganisms by direct methods in a paper given before the Society of American Bacteriologists in 1917. The next year (6) he gave the procedure in more detail and stated that it could be used in the study of soil bacteria and fungi "qualitatively as well as quantitatively". Fundamentally the procedure is a dry slide technique as follows. The soil is diluted (1/10 or greater depending upon the amount of clay the soil contains) with a dilute gelatin fixative prepared by dissolving 0.15 grams of gelatin in 1000 cubic centimeters of hot water. After the dilution is sufficiently shaken 0.1 cubic centimeter of the infusion, if quantitative work is being conducted, is spread on a clean glass slide in such a manner that it will evenly cover one square centimeter. The fixative is then dried by placing the slides over a water bath of boiling water where it dries quickly. After fixing the slide is stained. Conn tried a number of stains but suggested that rose Bengal in a phenol solution (1 gram of the dye in 100 cc of 5 per cent phenol) is best. The stain is added while the slide is still on the water bath and allowed to remain one minute after which it is washed "briefly by immersion in water, without allowing it to stay in the water an appreciable length of time". The slide is then dried and examined under a microscope using an oil immersion lens. "A good preparation should show the bacteria a deep pink or red, the mineral particles uncolored, some of the dead organic matter light pink but most of it either yellow or unstained".

Conn (8, 9) later amended the above procedure and claimed that the modification rendered the method more controllable. The modification is as follows:

"Make a suspension of soil in nine times its weight of an 0.015 per cent solution of gelatin. Smear a drop of this in a thin film on a slide. Dry on a flat surface over a boiling water bath. While still on the water bath, cover with the following staining fluid:

Rose Bengal.....1.00 gram
$CaCl_2$.....0.01 gram
5 per cent aqueous phenol, 100.00 cc.

"Allow it to stand for one minute. Wash as rapidly as possible in tap water. Dry and examine under the microscope. A rather high power is necessary....."

Conn recognized the limitations of this method even for qualitative work. He stated that the organisms observed cannot be isolated and examined in pure culture. There is some difficulty encountered in distinguishing bacterial cells from actinomycetes spores and bacteria from one another. In spite of these objections the method was a clear step forward in soil microbiological investigational work.

Other objections to the dry slide technique were later raised by workers using the method. Conn (7) then suggested a wet slide procedure, in which about 10 milligrams or less of soil is placed on a microscopic slide and mixed with two or three drops of water until the soil is well dispersed. A glass rod is dipped into a saturated aqueous solution of methylene blue or Löffler solution and the rod introduced into the drop of soil infusion on the slide. After thoroughly mixing, a cover slip is placed over the drop and examination carried out, using the high dry lens instead of an oil

immersion lens. A well prepared mount is one in which the stained infusion appears distinctly blue to the naked eye but under the microscope is only slightly tinted. A slide too heavily stained need not be discarded. By placing a drop of water on one side of the cover slip and touching the other side with a small roll of filter paper the stain can be removed.

Winogradsky (23, 24) criticized Conn's earlier technique in that the large grains of inorganic soil material stain yellow and hinder the proper examination of the slide. Consequently Winogradsky suggested a modification of Conn's procedure so elaborate that it could easily be regarded as a new method. Briefly, the modification consisted of shaking a definite amount of air-dried soil in water and after the soil had settled pouring the suspension into a centrifuge tube. Shaking of the sediment was repeated twice. Each time the suspension was poured into the same centrifuge tube. The liquid is then centrifuged and the supernatant poured into another tube. The sediment in the first centrifuge tube is again suspended in water and both tubes are spun again. Slides are prepared by taking a drop of each suspension and each sediment, drying and staining with extra erythrosine in five per cent phenol solution.

The procedure devised by Conn and Winogradsky were described by them as direct methods. Up to a point they were but in each case the natural structure of the soil is extensively disturbed. Waksman (21, 22) in 1916 proposed a "direct inoculation method" which was a distinct step toward the examination of the soil without disturbance of its natural structure. The procedure as given by Waksman is as follows:

> "A clump of soil, the size of a large pea, taken out carefully from the soil sample with as little contamination from the air as possible, is placed in the center of a sterile plate, into which

> 10-12 cubic centimeters of a sterile nutrient agar, favorable for the development of fungi has been placed; the soil is slightly pressed into the agar, so as to be surrounded by the nutrient medium. The plates are incubated at 25 to 30°C. At the end of 24 hours incubation, the plates are examined. Mold hyphae are then found to radiate out of the clump of soil into the surrounding medium. It is based upon the fact that the fungi present in the soil in the form of mycelium will grow at once into the medium, before the spores can germinate and develop hyphae. When a small piece of agar containing the growing mycelium, preferably a tip of a growing hyphae as far from the clump of soil as possible, is transferred upon a sterile slant of agar practically a pure culture of the particular fungus may be obtained."

Because this method involves the use of nutrient media the same objection as raised to the plate and dilution methods is operative, namely, that it is essentially selective.

None of the methods so far described permits of the examination of the soil population in its natural habitat. At most they could only be expected to give information as to the type and morphology of organisms present but not their natural arrangement. However, considerable advances in this direction have been made by the "buried slide" or "soil print" technique, proposed quite independently by two different workers, Rossi (19, 20) and Cholodny (2).

The procedure of Rossi was in fact two methods. The one consists of momentarily pressing microscopic slides against a freshly exposed soil surface, removing, drying, staining and examining the material which adheres to the slide. The other consists of burying a slide horizontally in the soil for a period of time, later removing, drying, staining and examining. Of the two methods Rossi believed the first to be more useful and the second was apparently overlooked by all workers, including Cholodny, who in 1930 published the following buried slide technique, only slightly different from that of Rossi. After digging a shallow trench with one undisturbed vertical

wall, a glass slide is pressed against the vertical side. One end of the slide is allowed to extend a little above the top of the soil. The slide is held in place by filling the trench behind the slide with soil. After one to three weeks the filled-in soil is removed and the slide is quickly pulled away leaving on it a soil film containing microorganisms. The slide is dried, stained and examined.

After publication by Cholodny of the buried slide technique the method became widely known and recognized as another step forward in the microbiological investigation of soil in situ. Conn (10) introduced the small modification of placing the soil under investigation in glass tumblers and incubating them in the laboratory in order better to control the various factors under investigation. In the same paper Conn indicates the potentialities of the method in studying the differences in the flora of various soils, the effect of fertilizer treatments and moisture changes on the flora and colony formation in pure culture.

Demeter and Mossel (11) used the Rossi-Cholodny buried slide technique in studying the changes in the soil microflora brought about by fertilizer treatment. Two comparable fertilizer experiments were carried out on different soils using potatoes and sugar beets as crops. They found that in addition to its simplicity the technique had several advantages, (1) the whole of the microflora and microfauna of the soil could be observed at the same time, (2) the technique permitted attempts to differentiate the morphology of the microorganisms in situ, (3) observations of associations of the different microscopic forms were possible, and (4) quantitative changes could be observed in the characteristic groups caused by such factors as moisture, temperature and nutrients. Among the disadvantages they listed were, (1)

the impossibility of isolating particular organisms for pure cultural study, (2) quantitative counts of the flora and fauna can only be rough estimations and (3) it is not possible to make any identification of bacteria by observation of their appearance on the slide. The authors also suggested the use of the Rossi-Cholodny technique in studying the appearance of the flora in sterilized soil inoculated with pure cultures and known mixtures of various organisms, in order better to interpret the information obtained from slides buried under field conditions.

In 1935 Meyer (17) made a study of the Rossi-Cholodny technique. He used sterilized soil which had been inoculated with various organisms. From the study of the buried slides he found that some bacteria failed to grow in sterilized soil, the growth of other bacteria was somewhat changed and in that of others no difference was noted.

Ziemiecka (25) in 1935 used the Rossi-Cholodny technique as modified by Conn to "study the physiogeny of microbial life in soils after disturbing the normal soil equilibrium by the addition of different single organic substances with a view to examining their chemotactical effect upon soil microbes". The results obtained pointed to the dominant role played by bacteria in the decomposition of certain of the more common organic compounds. They also showed the specific character of different groups of microorganisms, especially bacteria in the breakdown of particular organic substances in the soil.

The buried slide technique is definitely to be regarded as a direct method of soil investigation, although it has certain obvious defects. These defects have been at least partially overcome by a method suggested by Kubiëna (14, 15, 16) in 1932 which allows of direct microscopic examination

of the soil in an undisturbed state in the laboratory. It permits studies of the influence of materials on the soil and the isolation and transference of particular organisms direct to agar or gelatin plates. The amount of handling of the soil involved in the incorporation of other substances into it does not so permanently change its structure as does centrifuging or the making of a smear. With respect to this alteration Kubiëna and Renn (16) state, ".....under the influence of the physical, chemical and biological processes acting in it the soil is developing to a new, well-organized unit ....". The details of this procedure will not be given here as they are later described in the experimental section. The Kubiëna technique comes nearer to giving a complete picture of the microbial life of the soil than any other of the proposed "direct methods".

To supplement the "buried slide" observations and so as to be able to observe changes in the flora as conditions in the soil altered, Cholodny (3) in 1934 introduced his "soil chamber" method. In describing this method and comparing it with the buried slide method Cholodny said,

> "....that instead of a great quantity of soil, in which the object slide is buried, we have a comparatively small quantity of it, distributed as a thin layer of equal thickness between the object and cover slides. In this layer there may happen small hollows near the surface of both slides. Let us further assume, that one of these hollows has a depth equal to the thickness of the whole soil layer, from the cover to the object glass. We should have then a small moist chamber in the soil. When illuminating it through the object glass we could observe through the cover slide the organisms which develop inside. Such a transparent soil camera can be, of course, prepared also by artificial means."

The soil chamber preparations are made with dry sieved soil moistened just enough so the particles cling together but do not form a semi-liquid mass. The slides are incubated at room temperature in a dark space, saturated with water vapor.

The "soil chamber" method permits the isolation of organisms from the cover slip by means of micromanipulators, after removal from the artificial soil space. Alternatively the cover slip may be removed, dried and stained, being treated in fact, as a "buried slide". The obvious objection to this method when used for isolating microorganisms from the soil is that the culture is disturbed and future observations cannot be made. A slight modification of the method would circumvent this objection and permit isolations without destroying the structure of the culture.

A refinement of the "soil chamber" procedure is the "bodenstaub" method proposed by Cholodny (4) in 1936. The "bodenstaub" method fails as does the previous one in that the natural soil structure is disturbed but it does offer a means of studying relationships between soil organisms that the other methods are unable to give. In brief, the method as given by Cholodny is as follows: Air-dried soil is dusted on the center of a clean and flamed cover slip from a glass tube one end of which is closed with a thin screen of copper wire. A drop of sterile distilled water is placed in the depression of a sterilized bacteriological well slide and the cover slip, with the dusted side down, is then placed over the depression. The cover slip is sealed to the slide by adding a drop of sterile water to the edge of the cover slip; the water will move into the capillary space between the two. During incubation the evaporation of the water in the well and its subsequent condensation on the cover slip creates water films around the soil particles and it is in these water films that the microorganisms in the soil develop and may be observed.

EXPERIMENTAL

The work described in the following pages was an attempt to determine the characteristic microbiological features of two typical Iowa soils. As it is known that the addition of available organic matter to soils greatly influences microbial activity it was believed that any fundamental microorganic differences between the two soils under investigation might be brought out or emphasized in their behavior on addition of green manure. In addition to the examination of the soils themselves, observations were therefore made on samples into which organic matter, as green manure, had been incorporated.

Characterization along these lines would require more than a knowledge of the microorganisms present in the soils. It would be necessary to know if the organisms appeared in any definite succession and if their habitats differed. Neither of these could be determined by culture methods but only by direct observation. Such work calls for methods which do not destroy the natural soil structure. Only two of the direct methods considered fulfil this requirement. One is the method of Kubiëna (14, 15, 16) and the other the Rossi-Cholodny (19, 20, 2) buried slide technique. A third method, the so-called "bodenstaub" technique proposed by Cholodny (4), although it involved destruction of the initial soil structure was also used.

The Kubiena method proved to be more suited for the observation and study of the soil fungi than for any of the other groups of microorganisms. Since this procedure was in fact extensively employed in this work the fungi have perhaps received more consideration than the other groups. The buried slide method was used to obtain a picture of the interrelationships of the

different groups under field conditions and particularly to add to the information on the distribution of the soil bacteria. Finally the suitability and limitations of the "bodenstaub" method in investigations of this type were examined.

The information obtained through the use of these methods, though far from being complete, showed that distinct microbiological differences exist between the soils in question, and moreover, demonstrated that direct methods of observation are suitable for studying such differences.

Soils Used

Two soil types were used in the experimental work, one from the Mississippi loess area and the other from the Wisconsin drift area. The Tama silt loam, a widely distributed soil type of the loess area, was obtained in Marshall County about one and one-half miles northeast of State Center, Iowa. The Webster silt loam, a common soil in the drift area, was obtained eight miles north of the Iowa State College campus.

The Tama silt loam is an upland soil. Its surface is a dark brown moderately heavy silt loam, 10 to 12 inches deep (1). Below that is a layer of brown heavy silt loam about 4 inches thick which then grades into a brownish-yellow silty clay loam, the color becoming lighter with increasing depth. The subsoil is usually uniform, though often mottled with light gray and some streaks of rusty iron stains. The pH of the soil, as determined by the quinhydrone electrometric method, was about 5.6.

The Webster silt loam is an important soil type found in extensive areas on the uplands in the drift area. The surface of the type to an average depth of 13 inches is a very dark brown to black mellow silt loam (1).

The lower surface layer, to a depth of 17 inches, is a dark brown heavy silt loam. The soil varies in depth from 8 to 18 inches and may vary to a heavy silt loam. The subsurface soil, to a depth of 24 inches, is a brownish-gray to drab silty clay loam to silty clay. The color varies to dark gray, gray and dark grayish-brown. The texture may vary to a clay loam. The calcareous subsoil, to a depth of 34 inches, is a dark gray silty clay to clay mottled with gray, yellow, brown and rusty-brown. In topography the type is level to flat or depressed or gently sloping. It is mostly cultivated and when properly drained it is highly productive. The pH of the soil, as determined by the quinhydrone electrometric method, is about 6.6.

Additions Used

The soybeans and alfalfa employed as green manure in this study were grown upon the same soil type to which they were to be added. This was a precaution against introducing any foreign organisms into the soils under observation. The plants were gathered in sterile moisture chambers and then taken to the locations where the soil samples were collected. The percentages of nitrogen in the additions as determined by the Kjeldahl method were as follows: Alfalfa grown on Webster soil (young plants) 5.2, alfalfa grown on Tama soil, 2.6, soybeans grown on Webster soil, 2.9, and soybeans grown on Tama soil, 1.4.

For sake of brevity the word "addition" in the discussions which follow will imply the soybeans and alfalfa which were used as green manures in this work, for example, "soybean addition" will mean the cultures which contain soybeans mixed with the soil. "Soil alone" will imply the soil with nothing added to it and "soybeans alone" and "alfalfa alone" will mean those cultures which contain only soybeans and alfalfa.

Preparation of the Cultures

Kubiëna Technique

Sheets of sterile wrapping paper were unrolled and spread upon the ground. By means of large shears, sterilized over an alcohol lamp, the soybeans and alfalfa, each on a separate sheet, were cut into small pieces. Half an inch or so of the surface soil was scraped away from a small area and then some of the exposed soil gently crumbled with a large metal spoon sterilized over the alcohol lamp. One of the sterilized culture dishes was taken and filled loosely with soil. After determining in this way the amount of soil necessary for filling the dish, usually about 125 grams, the soil was emptied on another sheet of paper and mixed with some of the plant pieces. The amounts of alfalfa and soybeans mixed with the soil of each dish were approximately the amount which would be incorporated into the soil as green manure. Four dishes were filled with mixtures of soil and soybeans and four dishes with soil and alfalfa. Then three dishes were filled with soil alone and two each with soybean and alfalfa pieces, 15 dishes in all. Between every operation the tools were cleaned and resterilized over the alcohol lamp.

All the dishes were then brought into the laboratory. One of the dishes of soil was opened and while observing the surface through the soil microscope, water was added by means of a pipette until the primary, but not the secondary, soil spaces were filled with water. This amount of sterile distilled water was then added to each of the other dishes.

The culture dishes were Pyrex crystallizing dishes (See Plate I, Fig. 1). The smaller with a diameter of 70 mm and a height of 50 mm, served as the bottoms while larger dishes, with a diameter of 80 mm and a height of

40 mm, served as covers. The covers, supported by means of small brick-shaped pieces of cork held by rubber bands around the bottom dish, could be raised to any desired height above the soil in the dish. Excessive evaporation from the culture dishes was prevented by placing a strip of cotton wadding in the space between the cover and the bottom dish (See Plate I). After removal of the cover the cultures were examined through a soil microscope as described by Kubiëna (13) using either an oblique illuminator for lighting or an electric torch held by a ring stand (See Plate II).

The cultures were run in parallel. One dish of each series was temporarily opened in order to permit microscopic examination of the culture and to make isolations and transfers of the organisms to culture media by means of fine platinum or glass needles. The culture media used were Czapek's agar, potato-dextrose-agar and a beef extract-peptone agar. The other cultures were kept closed to prevent microbial contamination from the laboratory air. The dishes which were opened were used for observations during a period of about a week, after which another set of dishes was taken, the preceding set being discarded. In those series in which there were only two dishes, observations were made on each dish for almost two weeks before discarding them.

Direct isolation and transference to agar plates of small colonies of actinomycetes were often difficult without contamination with other organisms. Small soil particles often would be transferred with the colony and the bacterial contamination usually resulting would overgrow the actinomycetes. This difficulty was overcome in the following manner: The actinomycetes were picked up by means of a sterile needle and dislodged into a small amount of sterile water in a 8 cm by 1 cm test tube. After shaking thoroughly

the suspension was poured into a sterile petri dish and nutrient agar, sufficiently cooled, was poured into the plate. After the colonies of actinomycetes had developed it was a simple matter to obtain a pure culture transfer of the parent colony.

Rossi-Cholodny Technique

Shallow trenches with one vertical wall were dug in the soil. Clean, flamed slides were pressed against the vertical surface and the trench filled in behind the slide. In order to study the influence of the materials under investigation on the microbial activity of the soil the plants were cut in small pieces and mixed with the soil. The soil and plant residues were pressed against the slide and the whole covered with the soil which had been removed in digging the trench.

In the Tama silt loam four sets of slides were buried. One set was for the study of soil plus alfalfa, another the soil plus soybeans, the third was soil plus well-decomposed farmyard manure and the fourth was the check, the soil with no additions.

In the Webster silt loam only three sets of slides were buried. These were soil plus soybeans, soil plus sweet clover, and the check. Alfalfa was not available at the time the slides were buried in the Webster soil so sweet clover was substituted.

The slides were retrieved for examination one month after burying. The burying time was longer than that recommended by Cholodny since the soil was quite dry during the period. Only one small rain fell and that about the middle of the third week.

Galvanized iron sampling cylinders 6.5 cm in height and 8 cm in diameter were found useful for retrieving the slides. A cylinder was forced down its

complete height around a slide by the weight of the foot and by means of a trowel or large knife the cylinder was dug out of the ground. In this way the slide and its surrounding soil was brought undisturbed into the laboratory in a sterile moisture chamber and left intact until such a time as required for examination. This also presented an opportunity for observing the condition of the material which had been in contact with the slides.

"Bodenstaub" Technique

The "bodenstaub" technique as described by Cholodny was followed in this study except for two minor changes. In place of thick glass well-slides as suggested by Cholodny, ordinary glass slides, 3 inches by 1 inch, were used on which sections of glass tubing, 15 mm in diameter and 3 mm in height, were cemented with sealing wax (See Plate I, Fig. 2). The other modification concerned the dusting of the soil on the cover slips. Instead of using a glass tube to separate out the fine particles of the soil a glass rod heated until a knob had formed was dipped into the soil and then lightly tapped to dislodge the fine soil particles adhering.

Modified "Bodenstaub" Technique

A modification of the "bodenstaub" technique was used in the latter part of the study and found to be more valuable than the original method. Instead of utilizing dry soil, small soil particles containing decomposing fragments of alfalfa and soybeans taken from the Kubiëna cultures were employed.

PRELIMINARY WORK

In order to become familiar with the methods to be used in this study and to acquire an insight into the microbiological features of the soils preliminary studies were made on both the Webster and Tama silt loams.

The first of the introductory work was some field observations on a Webster silt loam in the early spring when the soil was still wet and cold. The examination disclosed two different types of decomposition taking place. In the poorly-drained, depressed areas the decomposition was almost entirely anaerobic, while in the better drained areas it was aerobic. Cornstalks taken from the damper soil bore no evidence of any fungous decomposition but frequently fruiting areas of Aspergillus were found on cornstalks taken from better drained places. However, cornstalks which had been under anaerobic conditions if incubated in an aerobic environment soon became covered with fungous mycelium.

In the pithy portion of the stalks procured from the depressed areas numerous white mites were found. Many protozoa were observed in small amounts of liquid taken from this region by means of capillary pipettes. Small brown pellets of mite excreta were always seen near the mites. These pellets were almost solid suggesting that the mites had been feeding upon some portion of the cornstalks. If this were true a change of food might be expected to result in some alteration in the excreta. A few of the mites were transferred to sterile agar plates and an examination of the plates 24 hours later disclosed a gradation in the excreta from the small, almost solid, brown pellets to larger, almost formless semi-liquid masses. In another but drier Webster soil both brown and black pellets were found in partially decomposed plant residues, although no mites were visible. These

observations imply that mites play a direct role in the decomposition of organic materials in the soil when sufficient moisture is present. The moisture content is an important controlling factor as evidenced by their gradual disappearance from the cornstalks as the stalks slowly dried out in the laboratory. This disappearance was also later noted in the Kubiëna cultures.

A few "bodenstaub" and soil chamber preparations were the only cultural studies made in the laboratory on the first Webster soil studied. In the soil chamber preparations some nematodes, a few rod-shaped bacteria and one non-septate and one septate fungus mycelium were observed. The septate mycelium was a Fusarium. The "bodenstaub" slides revealed a few bacteria around the soil particles and one non-septate mycelium. Further observations could not be made as the water evaporated and active growth ceased.

About one month after the field observations on the first Webster soil had been made another Webster silt loam was selected for examination. Practically no rain had fallen during the interim and the soil was quite dry. This field had been in oats the previous year and no cornstalk residues could be found as in the first field.

As Webster silt loam is a naturally poorly drained soil it is known that the first decomposition in the spring, except at the very surface, is anaerobic. At the time this second field was examined, however, most of the processes were aerobic. The plant residues were fairly well decomposed but in some cases their forms could still be determined and identification was possible in some instances. Pieces of plant residues were examined through the microscope and numerous protozoan cysts were found, especially in the interior of stems. None of the residues was moist enough to allow of the

separation of liquid from them as was previously done with cornstalks to discover if any active forms of protozoa were present. Many fungous hyphae and actinomycetes were found.

The fungi isolated from the Webster soil and their locations were as follows: Aspergillus niger, A. sulphureus and an unidentified Aspergillus were found on small root pieces; two cultures of Trichoderma were isolated, one from a piece of wood found buried in the soil and the other from a soil clump. A Cephalosporium was found on a soil particle and a species of Alternaria on a glume of grass. Spores closely resembling Diplodia spores, or some related genus, were found on a piece of an old cornstalk which had apparently been in the soil for two years.

Only "bodenstaub" cultural studies were made on this soil. A germinating Alternaria spore and a Fusarium fruiting were found in a preparation (See Plate III, Figs. 5 and 9). In water films surrounding mycelium numerous rod-shaped bacteria were observed (See Plate III, Fig. 1). Colonies of actinomycetes were seen (Plate III, Figs. 3, 4, 8) as well as numerous small elliptical spores, the appearance and size of which very closely resembled those of the genus Myrothecium, a fungus which was later found in both the Webster and Tama silt loam cultures. From one soil particle a septate mycelium, an actinomycetes and fungous spores were observed (See Plate III, Fig. 4).

All soil particles in the preparations were surrounded by films of water and after a few days most all these films contained bacteria (See Plate III, Fig. 2). Examination of the preparations of the Webster soil showed the same arrangements of the bacteria as noticed by Cholodny (4). Diagrammatic sketches of two of these arrangements are shown in Plate III,

Fig. 2 and 7). On one of the slides an Oospora developed (Plate III, Fig. 6). After incubating for about a week small protozoa were observed in the water films.

The preliminary work on the Tama silt loam differed from that on the Webster in that the cultural technique of Kubiena was used. Seven cultures were prepared as follows: Soybeans mixed with soil, sweet clover mixed with soil, manure mixed with soil, soil alone, soybeans alone, sweet clover alone and manure alone.

Twenty-four hours after the water had been added to the cultures a considerable development of fungous hyphae was found on the surface of the soybean and sweet clover addition cultures but only a little was found on the manure addition. Some colonies of actinomycetes were observed in all three of the above mentioned cultures. Among the fungi recognized in the cultures of soybean additions were a Helicoon, some colonies of Aspergillus, a Rhizopus and a Cephalosporium. The latter was also found in the culture of soil alone. In the sweet clover addition culture an Aspergillus and Cunninghamella were found.

It became evident even at the first examination that the manure culture was going to react differently from the green manure cultures. Sterile hyphae only were found in the manure addition culture. Nematodes were numerous and a few brown mites were present. In the cultures of the additions alone in absence of soil there was very little mycelium at first, although Alternaria was found on the soybeans. Some actinomycetes were present on the sweet clover and nematodes in the manure.

No great change in the flora as far as new genera were concerned was evident when observations were made six days later, due probably to the rapidity with which the cultures were drying out. The space between the

covers and bottoms of the culture dishes had not been packed with cotton to cut down evaporation as was later the case.

The preliminary work extended over a period of about two weeks. At the end of the period the soil culture and the cultures of soil mixed with the green manures and farmyard manure were broken open and examined. All had a wholesome earthy odor. In the cavities, if additions bordered on them, fruiting heads of Aspergillus and numerous colonies of actinomycetes were found. Within a week after the water had been added to the culture of sweet clover alone a strong putrefactive odor had developed but this odor became less intense as the leafy portion became more decomposed. The leafy portions of the sweet clover, excepting the midribs, had almost completely disappeared within 10 days. The decomposition apparently was nearly all accomplished by bacteria as few fungi were obvious.

In summing up the preliminary work the following salient points were brought out: (1) since the Webster silt loam is by nature a poorly drained soil the first decomposition in the spring, and during periods of considerable rain, is almost entirely anaerobic, except near the surface, (2) fungi are normally present in the Webster soil even in this condition, but are not active. Hyphae are observed soon after bringing the soil under aerobic conditions, (3) protozoa are present in an active state wherever there is sufficient moisture and food, (4) small mites are present and apparently play a considerable role in organic matter decomposition during periods of sufficient moisture content, (5) under aerobic conditions nematodes develop, (6) numerous species of fungi representative of many genera are present in the soil in a vegetative state, (7) the type of organic matter greatly influences the amount of fungous development, (8) well-decomposed farmyard

manure does not support as great a fungous development as additions of higher carbohydrate content, and (9) sweet clover decomposes far more rapidly than does soybeans.

KUBIËNA TECHNIQUE

Direct Microscopic Observations of the Tama Silt Loam Experiments

The preliminary work showed that owing to the rapid growth of the microorganisms during the first few days after setting up the cultures, it was not possible thoroughly to investigate seven cultures a day. As a result the main investigation was restricted to only five cultures, instead of seven as in the preliminary work. They were as follows: Soybean addition, alfalfa addition, soil alone, soybeans alone and alfalfa alone.

More than two weeks elapsed between the preliminary studies on the Tama soil and the commencement of the main work. As no rain had fallen during the time the soil was drier and 25 cc of sterile distilled water was necessary to bring the cultures to the required moisture content.

Progress Descriptions

1st Day. The additions exerted an immediate influence upon the activities of the microorganisms. This was shown by a greater abundance of hyphae in the addition cultures (especially near the additions) when compared with the soil alone. Rhizopus nigricans and Cunninghamella sporangia were seen.

One Rhizopus sporangium (See Plate IV, Fig. 3) was growing from the soil while another was developing from a stolon attached to a soybean leaf hair (See Plate V, Fig. 1). The Cunninghamella had originated in the soil of the alfalfa addition culture. A Helicoon colony was found on the soil of the soybean addition (See Plate IV, Fig. 1). A few colonies of actinomycetes appeared on residues and occasionally a colony was found growing on a soil particle.

The culture containing soil alone at this period showed very little

fungous activity. A few hyphae were observed in the cultures of soybeans and alfalfa alone.

The water which had been added to the cultures of soybean and alfalfa alone was turbid indicating that considerable bacterial development was taking place. The alfalfa alone cultures had a putrefactive odor not noticeable in the soybean cultures. This was probably due to proteolytic bacteria as the nitrogen content of the alfalfa was much higher than that of the soybeans.

3rd Day. All the cultures containing soil were drying out rapidly as the temperature in the laboratory was high.

Aspergillus was the next organism appearing in the soybean and alfalfa addition cultures. The fruiting areas of this genus practically covered every piece of residue upon which they were found. Three interesting observations were made at this time, namely, that Rhizopus produced nearly all of its sporangia from soil particles but most all the fruiting areas of Aspergillus originated from additions or pieces of organic matter already in the soil, and instead of Rhizopus nigricans producing 2, 3 or sometimes 5 sporangia from nodes on the stolons as in culture generally only 1 or occasionally 2 were produced. An examination of some of the particles from which the Rhizopus sporangia were produced revealed that while part of them were merely soil aggregates impregnated with solution others consisted of organic matter covered by soil particles.

Cephalosporium was found fruiting from the soil in both addition cultures. Fusarium was found on the alfalfa additions and Myrothecium and Trichothecium on the soybean additions. Colonies of actinomycetes continued to appear on both addition cultures. The soil by this time had dried con-

siderably and five cubic centimeters of water were added to the addition cultures. Very little change in the amount of hyphae visible took place but two Helicoon colonies and a few algal filaments were seen.

A few sterile hyphae were found on the fragments of soybeans and alfalfa alone and on the soybeans numerous Alternaria (See Plate V, Fig. 3) spores were seen and a large area of Acrothecium fruiting (See Plate IV, Fig. 2). No actinomycetes were found on either culture.

7th Day. Cephalosporium was becoming more abundant in the soybean addition cultures than in the alfalfa addition cultures. The fruiting areas of Aspergillus which so completely covered some of the additions at first were themselves becoming covered with hyphae of Fusarium. The same was true of Rhizopus, although this was not so conspicuous owing to the larger size of the Rhizopus sporangia. Cunninghamella of which only three or four sporangia were found had completely disappeared from the alfalfa additions. Acrothecium, however, was beginning to appear on the additions.

The culture of soil alone was becoming dry; there was no increase in the amount of hyphae present. Colonies of actinomycetes were frequently observed on both soil particles and plant fragments in the soil. After the observations were made 10 cc of water was added to the culture.

The culture of soybeans alone was thickly covered with hyphae of Fusarium, Alternaria and Trichothecium (Plate IV, Fig. 5). The surface of the alfalfa culture was covered with Monilia.

10th Day. By this time nearly all the Cephalosporium had disappeared from the soybean and alfalfa addition cultures and in both colonies of actinomycetes were becoming more numerous and larger. Monilia was found in the soybean addition. Mites, different from those under observation in the pre-

liminary work, were becoming abundant in the alfalfa additions.

The addition cultures had been under observation for ten days and the covers having been off so much during the examinations the soils had dried considerably. In addition so much exposure involved the possibility of microbial contamination.

As a new set of addition cultures was used for the next sequences of examinations the old cultures were placed on their sides and microscopic examinations were made through the glass of the dishes into the soil cavities opening against the walls. Similar observations were made through the bottoms of the dishes. In the large cavities, if additions were close or bordering on them, numerous fruiting bodies of Rhizopus and Aspergillus and many colonies of actinomycetes were found.

No important changes had taken place in the soybean and alfalfa cultures. Alternaria was still frequently found and Trichothecium was abundant. Often the conidiophores of the Trichothecium were so dense that the heads seemed to coalesce. In the alfalfa culture Monilia had covered nearly all the plant fragments.

14th Day. A number of Helicoon colonies were found in both soybean and alfalfa addition cultures but all attempts to obtain this organism in pure culture failed. A few Penicillium sporangia (Plate IV, Fig. 4) and a large number of mites and nematodes were observed in the soybean addition. These invertebrates play a very important part in the distribution of fungous spores through the soil. Nematodes and mites were more numerous in the fruiting areas of fungi than elsewhere. Many mites were so covered with spores that they seemed to have been dusted. An Aspergillus head was found upon the back of a mite; an exaggerated case of spore distribution by these

invertebrates.

At the time of this examination Monilia was frequently found on the alfalfa additions. However, from the observations on the additions thus far, Aspergillus was the most common fungus encountered, with Rhizopus and Cephalosporium following almost equal to each other.

19th Day. The cultures were drying out rapidly. No new fungi were observed but an Alternaria was seen in the soybean addition. Actinomycetes were apparently not suffering from the dry condition of the cultures but the mites and nematodes were disappearing. No changes were observed in the other three cultures.

Between the 19th day and the end of the experiment 25 days later, some observations were made but inasmuch as no changes were observed except the shedding of the fungus spores and the disappearance of the fauna from the surface of the cultures further discussion seems unnecessary.

On the 44th day some of the alfalfa residues were removed and macerated in a drop of water on a glass slide, stained and examined. Abundant protozoan cysts and some nematodes were found on the alfalfa leaf pieces. Droplets of water from the soil contained only a few protozoa but the water which was in contact with residues usually contained many large ciliates. The bacteria found were large chain-forming rods.

53rd Day. On the 53rd day at the close of the experiment an increase in Cephalosporium was the only noticeable change, although more water had been added on the 32nd day.

The alfalfa culture had decomposed into a slimy mass and lost its putrid odor but the soybeans had not decomposed as completely.

Examination of the old addition cultures disclosed that the additions were in various stages of decomposition more or less proportional to the age

of the cultures. Stem pieces of soybeans when split open revealed actinomycetes and filaments of fungi, the mycelium stretching across the hollow central portion of the stems (Plate V, Fig. 2). The following summarizes the results of the complete experiment.

(1) The soil of the cultures in all cases had a wholesome, earthy odor, as that noticeable in a newly plowed field.

(2) Except for the stems of the soybeans which were still hard and tough all residues showed considerable evidence of microbial action.

(3) The leaf pieces of the additions were in various stages of decomposition but in no case had they entirely disappeared.

(4) The alfalfa had decomposed more than the soybeans in the same length of time.

(5) Examination of most all residues disclosed colonies of fungi and actinomycetes.

Direct Microscopic Observations of the Webster Silt Loam Experiments

The cultures used in this portion of the work were similar to those used in the Tama experiments. It must be kept in mind, however, that the alfalfa used for the Webster experiments was younger and had a high nitrogen content while the soybeans were mature and the nitrogen content much lower.

Progress Descriptions

1st Day. This observation revealed an abundance of mycelium on the addition cultures in the vicinity of the additions and little in the soil away from the residues. This was confirmed by the culture of soil alone in which very few hyphae were found.

The alfalfa and soybeans alone contained some mycelium; Cladosporium and Alternaria were recognized.

2nd Day. This examination disclosed practically the same conditions as the first except that the alfalfa addition culture contained far more mycelium than did the soybean addition. The explanation for this probably lies in the fact that more leafy material was added in the case of the alfalfa than in the case of the soybeans and a wider nitrogen-carbon ratio existed.

3rd Day. On the alfalfa additions Rhizopus nigricans and Alternaria were found. Alternaria was always thickest on fibrous residues such as soybean stem pieces. Cladosporium was seen on both residues.

The culture of soil alone was interesting in that there was almost a complete lack of hyphae. This culture contained even less fungi than the corresponding Tama culture. With respect to the alfalfa and soybean additions alone, no new organisms were found but there was a considerable increase in hyphae.

5th Day. The surface of the alfalfa addition culture was almost completely covered with mycelium but the mycelium was heavier in some places than in others. Examination of these areas showed that wherever there was a piece of residue the fungous growth was heaviest. A Fusarium and Aspergillus were isolated from the culture.

The soybean additions failed to support as heavy a growth of fungi as did alfalfa but proportionately the growth was the same. A Fusarium was observed and Cladosporium and Alternaria were again seen.

There was no change in the culture of soil alone. The alfalfa alone had a putrefactive odor and Cladosporium was abundant on the alfalfa pieces.

Cladosporium and Trichotheeium were the only fungi found on the soybeans.

7th Day. In the soybean addition culture the fungous mycelium was still heavy and among the fungi recognized were Fusarium, Trichotheeium, Cladosporium and Alternaria. In the alfalfa addition culture were found Cladosporium, Alternaria, Trichotheeium and Fusarium, the last being by far the most abundant.

No change was noticeable in the soil with no additions. In the culture of alfalfa alone Cephalosporium was abundant, and a few Cladosporium heads were seen as well as some Alternaria and actinomycetes.

In the culture of soybeans alone Trichotheeium was so abundant on some plant fragments that the fragments could not be seen. On other pieces Monilia and Alternaria were found. A few actinomycetes were seen deep in the culture.

12th Day. Myrotheeium was found in both the soybean and the alfalfa addition cultures. Trichotheeium was observed again in both cultures growing from soil particles and plant residues.

The soil culture remained unchanged except for the presence of two algal filaments. The greatest change observed was in the cultures of soybean and alfalfa alone. In the soybean culture Trichotheeium had covered nearly every plant fragment while Monilia did the same in the alfalfa culture.

17th Day. By this time the surface growth on the addition cultures had apparently ceased. A few sporodochia were seen of what was believed to be Volutella on some alfalfa root pieces in the alfalfa addition culture. The soil culture had remained unchanged. In the soybean and alfalfa cultures the changes observed were the gradual disappearance of the fungi and decomposition of the plant pieces, the alfalfa decomposing more rapidly than the soybeans.

24th Day. The active flora in the cultures had apparently remained unchanged since the last observation. In the soybean and alfalfa addition cultures decomposition had not decreased in rate.

The examination of the cultures did not terminate at the 24th day, but as no apparent changes were evident except the disappearance of the mycelium further discussion will not be made.

Below are given the outstanding points brought out in all the cultures during the course of the experiment.

(1) In the addition cultures although an abundance of mycelium developed within the first 24 hours after the start of the experiment, fruiting of the fungi was not observed until the third day.

(2) The abundance of mycelium in the addition cultures was always greater in the vicinity of organic residues.

(3) The alfalfa addition cultures supported a heavier fungous growth than did the soybean addition cultures.

(4) Alternaria generally appeared more abundant on fibrous plant fragments.

(5) Webster silt loam had potentialities of great fungous activity but without additions of organic matter seems to be almost dormant.

(6) The cultures of alfalfa alone developed a putrefactive odor.

(7) In the culture of soybeans alone the genus Trichothecium predominated, whereas Monilia was the predominating genus in the corresponding alfalfa culture.

(8) The soil of the cultures in all cases had a wholesome, earthy odor as that noticeable in a newly plowed field.

ROSSI-CHOLODNY TECHNIQUE

The Rossi-Cholodny procedure was carried out as described in an earlier section of this paper.

Tama Silt Loam

Slides in soil alone

An examination of the buried slides revealed the same picture as was presented by the corresponding culture studies. No fungous hyphae were found on the slides and only traces of actinomycetes, these only where some organic matter already in the soil had been in contact with the slides.

The slides were a little richer with regard to bacteria, although the different kinds were few and their numbers small.

1. Coccus. 1.8μ diam. In vicinity of soil particles.
2. Rods. 2.2 by 1.8μ. " " " " "
3. Rods. 1.1 by 0.7μ. A large colony on some organic residue.

Slides in contact with alfalfa in the soil

These slides showed an abundance of fungi, actinomycetes and bacteria. This is in direct accord with the culture studies. Although bacteria were numerous on the slides there was a tendency for these microorganisms to be somewhat localized. This localization is not surprising if it is remembered that the soil had a pH of 5.6, a value a little below that which is considered optimum for the growth of most bacteria. It is a well-known fact that additions temporarily change the pH of a soil and that various microscopic locations in a soil may have different pH values. The locations of greater bacterial activity as shown on the slides probably have had a more favorable pH level for bacterial growth.

Partially decomposed spores of Alternaria were found on the slides. Other spores, some of which were probably Aspergillus and Rhizopus were also found. The spores and mycelium were always found in a mass with partially decomposed organic matter.

The various bacteria with their size and locations are listed below.

1. Rods. 1.3 to 1.4 by 0.7μ. Non-chain-forming. Along fungous hyphae; but not near other organic residue.

2. Rods. 0.8 by 0.6μ. Non-chain-forming. Along fungous hyphae.

3. Rods. 0.8 by 0.6μ. Chain-forming. Not near hyphae or other organic materials.

4. Rods. 0.7 by 0.5μ. In mass of organic matter, fungous hyphae and spores.

5. Rods. 2.2 by 0.9μ. Along mycelium. These rods were perpendicular to the long axis of the mycelium, whereas, the usual arrangement found was parallel.

6. Rods. 2.9 by 1.4μ. Non-chain-forming. Near organic matter and soil particles.

7. Rods. 4.2 by 0.5μ. Near soil particles.

8. Rods. 3.6 by 0.7μ. Chain-forming. Along almost completely decomposed fungous mycelium.

9. Rods. 2.8 by 0.7μ. Few. Along almost completely decomposed fungous mycelium.

10. Rods. 4.3 to 5.8 by 1.4 to 2.1μ. Found along fungous mycelium.

11. Rods. 2.5 by 0.7μ. Chain-forming. Not close to any organic matter.

12. Rods. 3.5 by 0.8μ. Along fungous mycelium.

The rods which occurred in the greatest numbers were about 0.8 by 0.6μ in size. These were found in every location where organic matter had been in contact with the slides. An examination of the distribution of the bacteria indicated that some of the microorganisms were associated with the decomposition of fungous hyphae as they were seldom found any distance from

these hyphae.

Another interesting observation was the presence of bacteria on fungous hyphae in various stages of decomposition. If the decomposition had not proceeded far the bacterial numbers were not great but they were directly proportional to the stage of decomposition until the material was practically gone after which the numbers again fell. Numerous cases were observed where fungi had entirely disappeared, and their previous locations were marked by faint outlines of the mycelium. In such places only a few bacteria remained.

Slides in contact with soybeans in the soil.

The same generalisations drawn from the preceding set of slides apply to the slides buried in contact with soybeans. Actinomycetes and fungi were numerous as determined by spore formation in the case of the former and the fungal mycelial development in the case of both. The lack of fungous spores on this set of slides may be explained by an observation made at the time of burying the slides. When mixing the alfalfa, soybeans and manure, especially the first two, with the soil, large cavities were present in the mixture, and some of these naturally opened against the surface of the slide. From the cultural studies an examination of such spaces revealed an abundance of fruiting fungi. Possibly this is an explanation of the fungous spores on the slides. But in the case with the soybean slides on which no spores were found another explanation needs be sought. This is found in the stage of decomposition of the material. As previously mentioned the soil at the time the slides were buried was dry and remained in that condition until about the middle of the third week when a small rain came. Upon breaking the soil away from the slides the soybean residues were found to be almost as they were when buried, practically no decomposition having taken place. Thus, although fungous mycelium was present, a stage of

abundant fructification had not been reached.

Although bacteria were not so numerous on these slides twelve different kinds, based upon size, were found. One of the twelve was a coccus form and the rest were rods.

1. Rods. 3.6 by 0.6μ. Scattered over locations where decomposing organic matter was in contact with the slides and near fungous hyphae.

2. Rods. 2.9 by 0.5μ. In the same locations as No. 1.

3. Rods. 1.8 by 0.7μ. Short chains. Scattered over the slides.

4. Rods. 3.6 by 1.6μ. Locations same as No. 1.

5. Rods. 1.4 by 0.7μ. Near soil particles. Formed colonies.

6. Rods. 3.6 by 1.1μ. Numerous around actinomycetes filaments but the arrangement indicated these not to be actinomycete spores. These rods were also numerous where organic matter had been in contact with the slides and also around decomposing mycelium.

7. Coccus. 1.4μ diam. Near soil particles.

8. Rods. 2.0 by 0.6μ. Numerous where organic matter was in contact with the slide.

9. Rods. 5.0 by 1.4μ. Numerous where organic matter was in contact with the slide.

10. Rods. 2.2 by 0.7μ. Numerous along mycelium.

11. Rods. 0.8 by 0.6μ. These predominate. Always found with the organic matter and decomposing mycelium.

12. Rods. 1.8 by 1.4μ. Colony formation. Near organic matter.

Slides in contact with well-decomposed farmyard manure in the soil

In accordance with some results obtained in the preliminary work an examination of the slides in contact with manure indicated that well-decomposed farmyard manure had little stimulating effect on fungi. Although no fungous hyphae were found a few spores and some filaments of actinomycetes

were seen. Five sizes of bacteria, all rods, were observed but their numbers were small. This may be accounted for as the manure was in an advanced stage of decomposition and available carbohydrate material, therefore, scanty.

1. Rods. 1.1 by 0.5μ. Chain-forming. On organic matter.

2. Rods. 1.4 by 1.0μ. Not near either organic matter or soil particles in some locations while in other locations they were found near these substances. These were the predominating organisms on these slides.

3. Rods. 1.3 by 0.7μ. Chain-forming. Numerous in spaces between residues of organic matter.

4. Rods. 2.2 by 0.6μ. Not near any organic matter residue.

5. Rods. 1.1 by 0.5μ. Colony formation close to soil particles, and also scattered over the slide.

Webster Silt Loam

Slides in soil alone

An examination of the check slides in the Webster soil revealed only a few fungi and no actinomycetes. Bacteria were more abundant both as to kinds and to numbers than in the case of check slides in the Tama soil.

1. Rods. 3.6 by 0.7μ. Scattered along mycelium.

2. Rods. 1.4 by 0.6μ. A few found near soil particles.

3. Rods. 1.0 by 0.7μ. Short chains. Where organic matter had been in contact with the slide.

4. Rods. 0.8 by 0.6μ. Always where organic matter had been in contact with the slide.

5. Rods. 2.2 by 0.7μ. Colonies around organic matter.

6. Rods. 1.8 by 0.7μ. Near soil particles.

7. Rods. 3.6 by 2.9μ. Colony near organic matter. Probably Azotobacter.

Slides in contact with sweet clover in the soil

Actinomycetes and fungi were not abundant on the slides in the Webster soil although the sweet clover had almost disappeared. Decomposition below the surface under the conditions often existing in the Webster silt loam is almost entirely anaerobic and bacterial. Under such conditions fungi would not be abundant. An examination of the slides revealed the bacteria to be numerous in total numbers but only eight different kinds based on size were found.

1. Rods. 1.0 by 0.7μ. Numerous. Found where sweet clover had been in contact with the slides.

2. Rods. 2.2 by 0.8μ. Few. Found where sweet clover had been in contact with the slides.

3. Rods. 4.3 by 0.9μ. Slightly curved. Found near organic matter attached to the slides.

4. Rods. 3.6 by 0.7μ. Found scattered over slides not near any organic residues.

5. Rods. 2.9 by 0.9μ. Colonies found near soil particles and near organic residues in advanced stages of decomposition.

6. Rods. 3.6 by 0.8μ. Slightly curved. Found scattered among the short plump rods which predominate.

7. Rods. 0.8 by 0.6μ. Numerous where there is or has been organic matter. These rods predominate.

8. Rods. 3.6 by 1.1μ. These were found where there was evidence that decomposing sweet clover had been in contact with the slides.

Slides in contact with soybeans in the soil

On slides in contact with soybeans in Webster soil numerous Alternaria and some Rhizopus spores were observed and also some spores which could not be identified. Mycelium was abundant on the slides but actinomycetes were seldom encountered. Bacteria were numerous, especially the short plump rods

0.8 by 0.6 which were the predominating organisms. Eight different sizes of bacterial rods were found.

1. Rods. 0.8 by 0.6μ. These predominate. Found with organic matter and mycelium.

2. Rods. 1.4 by 0.6μ. Along fungous hyphae.

3. Rods. 2.2 by 0.7μ. Along fungous hyphae.

4. Rods. 3.6 by 1.2μ. Distribution same as the smaller rods, 0.8 by 0.6μ, but the number was considerably smaller.

5. Rods. 1.8 by 0.9μ. Found where organic residues were not heavy, also along mycelium.

6. Rods. 1.1 by 0.4μ. Found along mycelium.

7. Rods. 2.9 by 0.7μ. Slightly curved. No definite location.

8. Rods. 2.9 by 0.7μ. With decomposing organic matter, especially decomposing mycelium. These rods were not curved.

After the examination of the slides had been completed the occurrence of bacteria of like size from the various slides were compared. On this basis it was found that, as might be expected, many of the groups appeared on more than one set of slides often in both soils, and their habitats were often identical.

1. Rods. 1.1 by 0.7μ. Formed colonies. Found in Tama soil and Tama soil + manure.

2. Rods. 1.1 by 0.7μ. No colony formation. In Webster soil + soybean and Webster soil + alfalfa.

3. Rods. 1.3 to 1.4 by 0.7μ. In Tama soil + alfalfa and Tama soil + manure.

4. Rods. 0.8 by 0.6μ. In Tama soil + alfalfa and Tama soil + soybeans; also in Webster soil and Webster soil + sweet clover and Webster soil + soybeans.

5. Rods. 2.2 by 0.9μ. In Tama soil + alfalfa, + soybeans and + manure; in Webster soil + sweet clover and Webster soil + soybeans.

6. Rods. 3.5 by 0.8μ. In Tama soil + alfalfa; in Webster soil and in Webster soil + sweet clover.

7. Rods. 2.9 by 0.5μ. In Tama soil + soybeans and in Webster soil + soybeans.

8. Rods. 1.8 by 0.7μ. In Webster soil and in Webster soil + soybeans.

9. Rods. 3.6 by 1.1μ. In Tama soil + soybeans. Webster soil + sweet clover and Webster soil + soybeans.

Observations on the Materials in Contact with the Buried Slides

After removing the slides from the soil the condition of the organic material which had been buried in contact with them was examined.

The manure buried in the Tama soil was in such an advanced stage of decomposition that it was difficult to determine on which side of the slides it had been placed, as the front and back of the slides had not been marked. No fungi were found in this material.

The soybean pieces were just beginning to show signs of decomposition. The edges of the fragments were decomposing but in the centers the structure was preserved and the green color unchanged. On some of the pieces sporodochia of Myrothecium and fruiting heads of Cladosporium were found.

The alfalfa was in a more advanced stage of decomposition than the soybeans, and the pieces had turned completely brown. A greater abundance of mycelium and fruiting bodies of Cephalosporium and Fusarium and an organism previously tentatively identified as Volutella were found. There were also numerous colonies of actinomycetes.

In the Webster soil the sweet clover was in an advanced stage of decomposition. Scattered mycelium but no fruiting bodies of fungi were observed. The soybeans had not decomposed but the leaves were entirely brown. A con-

siderable amount of mycelium was seen and the genera Fusarium and Myrothecium recognized. Colonies of actinomycetes were numerous.

"BODENSTAUB" TECHNIQUE

The procedure for making the "bodenstaub" cultures was discussed in a previous section of this paper and need not be repeated here. After preparation the cultures were incubated in a sterile moisture chamber in an atmosphere saturated with water vapor. Two examinations of the cultures were made; on the first and fourth days after preparation.

Tama Silt Loam

The first observations on the Tama silt loam revealed an abundance of actinomycetes. The mycelium had grown from a number of the soil particles on the cover slip and had extended almost to the very edge of the water film. A few fungous hyphae were also seen but in numbers and development they did not equal those of the actinomycetes. Besides the actinomycetes and fungi a few very small protozoan forms were observed.

Bacterial development was not great at this stage, either in numbers or kinds. In general bacteria were more numerous close the soil particles, though a few of the forms appeared in the spaces between the particles.

The examination after four days of incubation revealed nothing new except a greater development of both fungi and actinomycetes, the latter still being the more abundant. There was an increase in bacterial numbers and in types, as judged by size.

The first five bacteria given in the list below are those which were observed at the first examination of the slide. The rest of ths list is comprised of those which were found during the second examination.

1. Rods. 8.4 by 1.7μ . Slightly curved. Around soil particles.

2. Rods. 1.7 by 0.7μ . Abundant. Around soil particles and in surrounding water films.

3. Rods. 3.3 by 1.3μ. Scattered.

4. Rods. 2.5 by 0.8μ. Scattered.

5. Rods. 4.2 by 1.7μ. Scattered.

6. Rods. 5.0 by 1.0μ. Scattered in spaces between particles.

7. Rods. 2.5 by 1.3μ. Scattered in spaces between particles.

(Numbers 6 and 7 were found mixed together)

8. Rods. 1.7 by 1.3μ. Abundant in spaces.

9. Rods. 3.3 by 1.7μ. Numerous small colonies near soil particles. Indication of slime formation.

10. Rods. 1.8 by 1.5μ. Abundant and scattered.

11. Rods. 3.3 by 1.7μ. Evenly spaced in spaces, 10 to 15μ apart.

12. Rods. 8.4 by 1.7μ. Slightly curved. Rods scattered through spaces.

13. Rods. 1.7 by 1.3μ. Around soil particles. Evenly spaced about 6 to 7μ apart.

14. Rods. 1.7 by 1.4μ. Formed small colonies. Colonies numerous.

Webster Silt Loam

The first observation on the Webster silt loam cultures revealed a smaller development of actinomycetes than in the Tama silt loam, and no fungous hyphae. The numbers of bacteria were apparently slightly greater yet only six different kinds, separated on the basis of size were found. The examination of the slides on the fourth day disclosed nothing new, except the presence of a few small protozoa and a slightly greater development of the actinomycetes.

In the following list the first six bacteria were observed at the first examination and the remainder are those observed at the second.

1. Rods. 2.5 by 1.7μ. Near soil particles.

2. Rods.	5.0 by 1.7μ.	Near soil particles.
3. Rods.	1.7 by 0.8μ.	Abundant over slide. Heaviest near soil particles.
4. Rods.	3.3 by 1.7μ.	Numerous in spaces between soil particles.
5. Rods.	3.3 by 1.7μ.	In small scattered groups.
6. Rods.	3.3 by 1.7μ.	Radiating from soil particles.
7. Rods.	1.7 by 0.8μ.	Small colonies. Congregated in spaces around soil particles. Numerous. Individuals so close together they seemed to coalesce.
8. Rods.	4.2 by 1.3μ.	Evenly spaced about 7μ apart. Abundant. These rods predominate.
9. Rods.	2.5 by 2.0μ.	Abundant in spaces between particles.
10. Rods.	3.3 by 1.3μ.	Abundant. Irregularly spaced but well distributed.
11. Rods.	2.6 by 1.7μ.	Evenly spaced about 6μ apart.
12. Rods.	3.3 by 1.7μ.	Indication of chain-formation, and of antagonism as other organisms surrounded these but did not develop close to them.

MODIFICATION OF THE "BODENSTAUB" TECHNIQUE

The modification of the "bodenstaub" technique discussed earlier was carried out along with the regular "bodenstaub" studies, under the same conditions.

Tama Silt Loam

Alfalfa

The first observations on the decomposing alfalfa fragments disclosed numerous protozoa, actinomycetes and fungi. The actinomycetes and fungi generally developed from the particle of soil containing the alfalfa residue but some were developing from smaller soil particles which broke away from the parent particle. The examination on the fourth day disclosed only a greater development of the microbial forms and a few more bacteria, based on size. The only fungus which had fruited was a Penicillium.

The bacteria developed in numbers rapidly between the first and second examination, although a total of only 12 different kinds was observed. At the second observation two types seemed to predominate, one consisted of large rods which were well scattered and the other was a slime producing rod, the individuals of which were so close together that their complete outlines could not be distinguished. The following list is made up of the different bacteria observed; the first six being those found at the first observation and the remainder those found at the second.

1. Rods. 3.3 by 1.2μ. Found in spaces a considerable distance from any organic matter.

2. Coccus. 1.7μ dia. Small colonies. Not near any organic matter.

3. Rods. 2.5 by 1.0μ. Slightly curved. Formed relatively curved chains.

4. Coccus. 2.5μ. dia. Small colonies, numerous near organic matter.

5. Rods. 5.0 by 1.2μ. Slightly curved. Not near any organic matter.

6. Rods. 6.5 by 1.5μ. In water films along fungous hyphae.

7. Rods. 2.0 by 0.8μ. Small colonies in spaces.

8. Rods. 3.3 by 1.2μ. Slightly curved. Scattered.

9. Rods. 3.3 by 1.7μ. Formed short curved or sharply angular chains. Chains scattered.

10. Rods. 5.0 by 1.7μ. Slightly curved. Scattered in spaces.

11. Rods. 4.2 by 1.4μ. Abundant and scattered.

12. Slime producing rods forming a network in spaces.

Soybeans

The soybeans in the Tama silt loam cultures contained only a few colonies of actinomycetes at the first observation, no fungi, a few protozoa and only four kinds of bacteria. At the next observation an abundant development of actinomycetes was noted, but only a few scattered fungous hyphae, no noticeable increase in protozoa, and only three additional kinds of bacteria.

Following is a list of the bacteria found in the soybean cultures.

1. Rods. 3.5 by 1.7μ. Near organic matter.

2. Coccus. 2.5μ dia. Abundant, near organic matter.

3. Rods. 1.7 by 0.8μ. Small colonies near organic matter.

4. Rods. 5.0 by 1.5μ. Scattered in the spaces.

5. Rod. 2.0 by 1.4μ. Abundant and scattered.

6. Rods. 3.3 by 1.7μ. Form large colonies.

7. Rods coalesced, colonies spreading, individuals indistinct.

Webster Silt Loam

Alfalfa

The same generalizations which were given for the alfalfa in the Tama silt loam apply to the alfalfa in the Webster silt loam. The type and manner of growth of the microorganisms were apparently similar.

Fungi and actinomycetes were numerous even at the first observation as well as protozoa and bacteria. The bacteria were abundant and six kinds were found. The second observation disclosed further development of actinomycetes, bacteria, protozoa and a few nematodes. Only two fungi were found, namely, Fusarium and Cephalosporium. The following list gives the six bacteria observed in the first examination followed by the nine observed during the second.

1. Rods. 2.5 by 0.8μ. Radiated from soil particles.
2. Rods. 3.3 by 1.3μ. In water film along fungous hyphae.
3. Rods. 3.3 by 1.3μ. Short chains. In spaces between fungous hyphae.
4. Rods. 2.5 by 1.2μ. Short chains. In spaces.
5. Rods. 3.8 by 1.4μ. On residue.
6. Coccus. 2.1μ dia. Along fungous mycelium.
7. Rods. 3.3 by 1.0μ. In water film along fungous hyphae. Motile.
8. Rods. 3.3 by 1.0μ. Non-motile. In water film along hyphae.
9. Coccus. 1.5μ dia. Small scattered colonies.
10. Rods. 5.0 by 1.3μ. Numerous. Scattered.
11. Rods. 6.5 by 1.7μ. Numerous. Scattered.
12. Rods. Actively motile, along mycelium. Measurement impossible.
13. Large areas of slime-forming bacteria. The individuals could not be distinguished.
14. Coccus. 1.7μ dia. Abundant and scattered.

15. Rods. 1.7 by 1.2 . Scattered.

Soybeans

The soybean cultures in the Webster soil showed about the same picture as soybeans in the Tama soil. There were some actinomycetes and fewer fungi. No protozoa were observed and only three different kinds of bacteria. Conditions were not greatly different at the second examination. A few small protozoan forms were found and the fungi had fruited, making identification possible. Two genera were found, namely, Fusarium and Hormodendrum*. Six bacteria were found.

1. Rods. 2.1 by 1.7μ . Scattered.

2. Coccus. 2.1μ diam. Along fungous hyphae.

3. Rods. 5.0 by 1.2μ . Numerous. Very slightly curved.

4. Rods. 2.5 by 0.8μ . In spaces between particles and around organic matter.

5. Rods. 3.3 by 1.0μ . In spaces between particles and around organic matter.

6. Rods. 5.0 by 1.7μ . Abundant around organic matter.

7. Rods. 1.3 by 1.0μ . Abundant around organic matter.

8. Rods. 3.3 by 0.8μ . Scattered.

9. Rods. 2.5 by 1.0μ . Indication of chain formation. Probable antagonistic effect as other forms failed to develop near this organisms but did form all around the short chains.

*There was some doubt in the identification of this organism, which might have been Cladosporium. The main difference between these organisms is that the spores of the Cladosporium are two-cells, while those of Hormodendrum are single cells.

In this work the protozoa were not studied thoroughly but the forms on one set of "modified bodenstaub" slides were identified. All were common soil forms, namely, Cercomonas longicauda, Nuclearia, Bodo saltans, Colpoda and Vahlkampfia. Throughout this work the forms most commonly observed belonged to the genera Colpoda and Cercomonas, the former being the more numerous. The Colpoda and Cercomonas forms were generally observed whenever water taken from near fragments of alfalfa and soybeans in the addition cultures was examined through the microscope, although they were more frequently found near the alfalfa.

DISCUSSION

Interpretation of Results

Any attempt to determine the characteristic microbiological features of soil should include not only the determination of the organisms present but their morphology *in situ* and the interrelationships between the groups. It should also include observations on the potentialities of the soil population and the sequence of active forms within it when confronted with a major change in the status of the soil organic matter, such as is produced by green manuring or the addition of farmyard manure.

No one method has been devised capable of giving this information simply, and accordingly it is necessary to build it up from observations taken in a number of supplementary procedures.

The examination in the preliminary work of cornstalks obtained from wet depressed areas and drier areas in the Webster soil brought out the fact that in this soil two different types of decomposition were taking place. In the former areas the almost total absence of fungous mycelium and the soggy condition of the stalks were indicative of anaerobic conditions, while the stalks from the drier areas had a much lower moisture content and contained an abundance of mycelium and many fruiting heads all of which pointed to aerobic conditions. That there were fungi present in the stalks from the depressed areas, though in an inactive or dormant condition, was shown by the abundance of mycelium developing on the soil and stalks within a few days of culturing in the laboratory under aerobic conditions.

From the numerous mites found in the pithy portion of the cornstalks it is inferred that these invertebrates have some role in the decomposition of plant residues in the soil. Although it is possible that they feed upon

some soluble materials present or upon microbial tissues, the change in their excreta when they were transferred to a nutrient agar plate would indicate that their food in the cornstalks was of a solid nature.

In the Tama soil, unlike the Webster soil, colonies of actinomycetes were found on most of the organic residues examined, and these organisms were particularly obvious later in the Kubiena cultures. It has been suggested that the actinomycetes play a dominant role in the decomposition of grass roots. If that be true an abundance of actinomycetes would be expected in this particular field of Tama soil as it had been in pasture for the previous ten years.

The addition of alfalfa and soybeans to both the Webster and Tama silt loams caused a great increase of fungous activity. An interesting feature of this increase was the successions of the fungi, which, though not identical in both soils nor in one soil with both additions, had distinct resemblances. Tables I, II, III, and IV show these successions diagrammatically, recording in each case the first appearance of the organisms.

Apart from the succession of the fungi other interesting features brought out by the Kubiëna cultures were the differences between the soil forms and culture forms of Rhizopus, and the different fruiting habitats of Aspergillus and Rhizopus. The soil form of Rhizopus generally produced only one, but occasionally two sporangiophores from a stolon node, whereas, in agar culture two, three and sometimes five sporangiophores were seen. These sporangiophores were generally developed from particles of organic matter covered with soil and often from soil particles infiltrated with soil solution. On the other hand, the fruiting heads of Aspergillus generally developed from the plant additions.

Succession of Fungi in the Kubiëna Addition Cultures.

(Only the first appearance of the organisms is recorded).

Table I

Soybeans Added to Tama Silt Loam

Fungus	Days after start of experiment: 1	3	7	10	14	19
Rhizopus	X					
Helicoon	X					
Aspergillus		X				
Myrothecium		X				
Trichothecium		X				
Cephalosporium		X				
Fusarium			X			
Monilia				X		
Penicillium					X	
Alternaria						X

Table II

Soybeans Added to Webster Silt Loam

Fungus	Days after start of experiment: 3	5	7	12
Cladosporium	X			
Alternaria		X		
Fusarium		X		
Trichothecium			X	
Myrothecium				X

TABLE III

Alfalfa Added to Tama Silt Loam

Fungus	Days after start of experiment: 1	3	7	10	14
Cunninghamella	X				
Aspergillus		X			
Cephalosporium		X			
Fusarium		X			
Acrothecium			X		
Rhizopus				X	
Helicoon					X
Monilia					X

Table IV

Alfalfa Added to Webster Silt Loam

Fungus	Days after start of experiment: 3	5	7	12	17
Rhizopus	X				
Alternaria	X				
Cladosporium	X				
Fusarium		X			
Aspergillus		X			
Trichothecium			X		
Myrothecium				X	
Volutella					X

A noticeable difference between the two soils was brought out in the Kubiëna cultures which had received alfalfa and soybeans as additions. In each soil during the first few days there was a rapid development of fungi possibly more in the Webster than in the Tama, this being followed by a decrease in abundance of new hyphae. This decrease was slow and extended over a considerable period of time in the Tama soil, while in the Webster soil there appeared to be an almost complete cessation of fungous development after the seventh to the twelfth day.

The explanation for this almost sudden cessation of growth in the Webster soil in comparison with the Tama may lie in the different physical characteristics of the two soils. As the Webster silt loam is naturally a heavy poorly-drained soil, the addition of green manure and farmyard manure probably greatly influences the physical structure of the soil. Such additions to the Tama silt loam, a better drained and looser soil, would exert a much less noticeable influence. Such an alteration in the Webster soil, together with the addition of food materials would create a better environment for the growth of fungi, thus accounting for the abundance of these organisms in the Kubiëna addition cultures and the apparent lack of fungi in the Kubiëna cultures of soil alone. The softening and decomposition of the additions would result in a slow collapse of their structure allowing the soil to return to a condition almost as it was originally. In the case of the Webster this return would be to a more compact anaerobic condition unfavorable to fungi, whereas, in the Tama, with its naturally loose structure, the disappearance of the fungi would not be due to anaerobic conditions but to a disappearance of food materials. This would result in a gradual slowing of the fungous activity as compared with the almost sudden cessation in

the case of the Webster.

The information acquired from the buried slides was practically the same as that from the Kubiéna cultures, except that the observation of bacteria was possible and their distribution could therefore be followed. Green manure in contact with the slides resulted in the development of an abundance of fungi, bacteria and actinomycetes upon them. One exception to this was the sweet clover mixed with Webster soil. This did not result in an abundance of mycelium on the slides, but instead the appearance of large numbers of bacteria. In many cases, however, the distribution of the bacteria was such as to suggest that some of the groups were concerned more with the breakdown of previously elaborated fungous tissue than with that of plant additions.

Discussion of the Methods Employed

Even the best of the laboratory methods of soil investigation involve certain changed conditions in the soil which may not be comparable to field conditions, although the initial soil structure may be only slightly disturbed in obtaining the samples. In order to supplement the laboratory observations field investigations should, if possible, be run parallel with the laboratory study, though this is not always done in microbiological investigations of the soil.

The type of field observation to select will depend both upon the requirements of the study and the methods to be used in the laboratory. If the Kubiéna technique is chosen as the main laboratory procedure, the field investigations should consist of the microscopic examination of the soil and residues in the soil, and the use of the Rossi-Cholodny buried slide technique.

Investigations of small soil clumps and plant residues may reveal to the investigator many things helpful in the correct interpretation of the laboratory studies.

The results obtained by the Kubiena technique were in accord with those obtained from the field examinations in the preliminary work. True, all the organisms observed in the laboratory cultures were not found in the course of the field observations but the field observations cannot be so intensively conducted. In an examination of the alfalfa and soybean residues which had been in contact with the buried slides in this work, fruiting heads of Cladosporium, Cephalosporium and Fusarium and sporodochia of Myrothecium and Volutella were found, all of which had also been seen in the Kubiena cultures. Besides representatives of these genera many colonies of actinomycetes were found in both the cultures and on residues in the field. This was especially true in the case of the Tama soil.

In the examination of the Kubiena cultures care was taken not to keep the covers off the dishes longer than necessary, a precaution against contamination from laboratory air. After having served for observations over a period of time some of the cultures were opened and handled without aseptic precautions. It was interesting to find that no fungi appeared in these cultures which were not present in those cultures which had remained closed.

The Rossi-Cholodny buried slide technique is in reality a field method and it is capable of giving data comparable to that obtained from the Kubiena cultures. It also gives a better picture of the bacteria than any of the other methods used in this study. Owing to their small size any examination of the bacteria by direct methods is almost impossible; however, if the buried slide technique is used with other direct methods of investigation as was

the case in this work a fairly comprehensive picture of some of the interrelationships of this group of organisms with other groups can be obtained. The method also seems capable of revealing certain characteristic differences in the microbial populations of different soils, and when carried out in conjunction with the Kubiëna method is very helpful in giving a clearer picture of microbial activities and distribution in the soil.

The "bodenstaub" method appears to have limitations in that the original structure of the soil is completely destroyed, and the conditions so changed that the organisms developing may not truly represent the natural flora. It does, however, permit of pure culture decomposition studies on a microscale and single cell isolations if certain modifications of the well slide are made.

The modification of the "bodenstaub" technique like the original method, has limitations in that the original structure of the soil is destroyed but it is capable of giving much useful information particularly with respect to the soil protozoa, though the conditions probably favor this group.

SUMMARY

1. The Webster and Tama silt loams were alike in that in each the fungous activity was increased when soybeans and alfalfa were added to the soil. More genera of fungi, as determined by mycelial growth and fruiting, appeared in the Tama soil; however, the fungous activity decreased at a slower rate in this soil than in the Webster. In both cases a definite succession of fungi following the addition of green manure was observed.

2. The Webster silt loam is a heavy compact soil and most of the decomposition taking place within it is normally bacterial and anaerobic in wet periods, except at the very surface. On the addition of green manure, however, the structure may be so opened that aerobic conditions temporarily prevail. As the plant residues disappear the decomposition may revert to the anaerobic form.

3. Aspergillus and Rhizopus showed a difference in their fruiting habitats, the former fruiting generally on fragments of plants added as green manure and the latter usually directly from the soil. The soil form of Rhizopus differed from the culture form with respect to the number of sporangiophores.

4. Certain groups of bacteria seemed from their distribution to be almost entirely engaged in the decomposition of fungous tissue in the soil, whereas, others were active in the decomposition of plant residues.

5. The numerous mites found in the pithy region of the cornstalks buried in the Webster silt loam indicated that these invertebrates may be important in the decomposition of plant residues.

6. The protozoa always seemed more abundant in soil solution in direct contact with green manure than in contact with soil particles.

7. In a study of the microbiological features of soils it was found that direct methods of observation supplemented by field investigations are necessary in order to learn the arrangement of the microorganisms in the soil, their habitats, group interrelationships and the differences between soil forms and culture forms.

8. Laboratory methods, such as the Kubiëna technique, which do not completely destroy the initial structure of the soil, more nearly simulate field conditions and yield information comparable to that obtained by field observations. Such methods also permit of the study of fungous successions which are difficult to follow in the field.

9. The Rossi-Cholodny method offers a means of studying the influence of materials, such as green manures, farmyard manure and the like, added to the soil and at the same time preserving the natural habitats of the organisms acting upon such materials. The method also is capable of showing the arrangement and distribution of bacteria.

10. The "bodenstaub" procedure and its modification have such limitations that their value as direct methods of soil investigation are questioned, although they may be used in protozoan studies.

APPENDIX

Fungi Identified in the Kubiëna Culture

Tama Silt Loam

Alfalfa Additions

1. Acrothecium robustum Gilman and Abbott
2. Aspergillus Sp. 1
3. Aspergillus Sp. 2
4. Aspergillus fumigatus Fres.
5. Cephalosporium curtipes Sacc.
6. Cunninghamella sp.
7. Fusarium sp. 1
8. Helicoon sp.
9. Monilia acremonium Delacroix
10. Rhizopus nigricans Ehrenb.

Soybean Additions

1. Alternaria fasciculata Cooke and Ellis
2. Aspergillus fumigatus Fres.
3. Aspergillus sp. 1
4. Cephalosporium curtipes Sacc.
5. Fusarium sp.
6. Helicoon sp.
7. Monilia acremonium Delacroix
8. Myrothecium roridum Tode
10. Penicillium sp.
11. Rhizopus nigricans Ehrenb.
12. Trichoderma lignorum (Tode) Harz

Soil Alone

1. Helicoon sp.
2. Rhizopus nigricans Ehrenb.

Soybeans Alone

1. Acrothecium robustum Gilman and Abbott
2. Alternaria fasciculata Cooke and Ellis
3. Fusarium sp.
4. Trichothecium roseum Link.

Alfalfa Alone

1. Alternaria fasciculata Cooke and Ellis
2. Monilia acremonium Delacroix
3. Trichothecium roseum Link

Webster Silt Loam

Alfalfa Additions

1. Alternaria fasciculata Cooke and Ellis
2. Aspergillus fumigatus Fres.
3. Cladosporium epiphyllum Persoon
4. Fusarium sp.
5. Myrothecium roridum Tode
6. Rhizopus nigricans Ehrenb.
7. Trichothecium roseum Link
8. Volutella sp. (?)

Soybean Additions

1. Alternaria fasciculata Cooke and Ellis
2. Cladosporium epiphyllum Persoon
3. Fusarium sp.
4. Myrothecium roridum Tode
5. Trichothecium roseum Link

Soil Alone

No isolations

Soybean Alone

1. Alternaria fasciculata Cooke and Ellis
2. Cladosporium epiphyllum Persoon
3. Fusarium sp.
4. Myrothecium roridum Tode
5. Trichothecium roseum Link

Alfalfa Alone

1. Alternaria fasciculata Cooke and Ellis
2. Cephalosporium curtipes Sacc.
3. Cladosporium epiphyllum Persoon
4. Monilia acremonium Delacroix

Description of the Culture and Soil Forms of the Fungi Found in the Kubiena Cultures

Acrotheciun Preuss 1851 (12)

Hyphae creeping, slightly raised. Conidiophores erect, undivided, dark colored. Conidia long or spindle-shaped, 3- or more celled, colored or almost hyaline forming a terminal head.

Acrotheciun robustum (12)

Culture Form. Colonies on Czapek's agar broadly spreading, velvety, consisting mostly of submerged mycelium and aerial conidiophores, with little aerial mycelium; surface black, reverse black. Conidiophores arise from submerged or aerial hyphae, multi-septate, dark colored, thick-walled, smooth, 50 to 150μ long, averaging about 100μ. Conidia borne typically in terminal heads, but are occasionally produced laterally on the conidiophores; apex of the conidiophores very slightly inflated. Conidia elongate, barrel-shaped, 4- or 5-septate, thick-walled, dark colored, smooth, 37 to 50μ by 10 to 14μ.

Soil Form. The fruiting heads of 4 to 6 spores appeared brown to shiny black with incident light. The conidiophores were erect and were either well spaced or so close together that the spores seemed to coalesce, forming almost a solid brownish-black canopy over the substrate.

Alternaria Nees 1817 (12)

Sterile hyphae creeping, septate. Conidiophores single or in groups, erect, septate, mostly unbranched, short. Conidia inverted, club-shaped, mostly elongate at the tip, muriform in the lower portion, dark colored, lighter at the points, borne in more or less long, usually simple chains.

Alternaria fasciculata Cooke and Ellis (12)

Culture Form. Conidiophores brown, erect or ascending, irregularly curved, solitary or caespitose, septate, diameter uniform, 40 to 130μ by 3μ; conidia dark brown, oblong ovate, minutely apiculate, 35 to 90μ by 9 to 14μ, endochrome transversely two to seven times septate with usually several longitudinal septa, the apical cell short or elongated into a straight hyaline beak.

Soil Form. The dark colored spores borne in chains could readily be recognized with incident light. The conidiophores were erect but the spore chains bent over and were more or less decumbent.

Aspergillus Micheli 1729 (Corda 1840) (12)

Vegetative mycelium consisting of septate branching hyphae, colorless; conidial apparatus developed as stalks and heads from specialized enlarged, thick-walled hyphal cells (the foot-cells) producing stalks (conidiophores) as branches approximately perpendicular to the long axis of the foot-cells; conidiophores unseptate or septate usually enlarging upward and broadening into elliptical, hemispherical, or globose fertile vesicles bearing sterigmata, either parallel and clustered in terminal groups or radiating from the entire surface; sterigmata in one series, or as a primary series, each bearing a cluster of two to several secondary sterigmata at the apex; conidia varying greatly in color, size, shape, and markings, successively cut off from the tips of the sterigmata by cross-walls, and forming unbranched chains arranged into radiate heads or packed into columnar masses; perithecia found in certain species only; sclerotia regularly found in some strains, occasionally found in others.

Aspergillus fumigatus Fresenius 1863 (12)

Culture Form. Colonies on Czapek's agar in some strains strictly velvety, in others with varying amounts of tufted aerial mycelium up to felted floccose forms, green to dark green, becoming almost black in age, spreading. Reverse and substratum, colorless to yellow. Conidiophores short, usually densely crowded, up to 300μ(occasionally 500μ), by 2 to 8μ in diameter, arising directly from submerged hyphae or as branches from aerial hyphae, septate or non-septate, gradually enlarged, upward, with apical flask-shaped vesicles up to 20 to 30μ in diameter, fertile usually only on the upper half, bearing sterigmata in one series, usually 6 to 8 by 2 to 3μ, crowded, closely packed, with axis roughly parallel to axis of the stalk; chains of conidia form solid columns up to 400 by 50μ; conidia dark green in mass, globose, 2 to 3.5μ, mostly 2.5 to 3μ.

Soil Form. Conidiophores usually short, densely crowded, generally developed from additions of organic matter in the soil. Heads columnar; light green.

Aspergillus Sp. 1

Culture Form. Colonies on Czapek's agar were almost powdery and sulphur yellow, becoming almost green in old cultures. Stalks smooth. Heads radiate.

Soil Form. Conidiophores erect, scanty, with radiating conidial chains at apex. Heads distinctly yellow.

Aspergillus Sp. 2

Culture Form. Colonies in Czapek's agar spreading, floccose. Vegetative hyphae creeping, septate, hyaline; surface white, reverse colorless. Conidiophores sparsely produced. Heads radiate.

Soil Form. Heads radiate, white. Conidiophores scanty.

Cephalosporium Corda 1839 (12)

Sterile hyphae creeping. Conidiophores arise as short branches of aerial hyphae, erect, non-septate, not swollen at the tip. Conidia borne singly at the tips of the conidiophores, being pushed to the side as they are formed successively, not falling away, but forming a transparent head enveloped by slime; conidia usually ovoid, hyaline or slightly colored.

Cephalosporium curtipes Sacc. (12)

Culture Form. Colonies on Czapek's agar spreading, felty to floccose, pure white, consisting of creeping, septate, dichotomously branched hyphae, reverse colorless. Conidiophores arise as branches of aerial hyphae; short, up to 25μ long. Conidial heads round. Conidia elongate elliptical, hyaline, 9.0 to 10.0 by 3.5 to 4.0μ.

Soil Form. This genus was often difficult to distinguish from the genus Fusarium and positive identification was possible only by isolation and examination of spores by means of high power and transmitted light. The conidia did not fall from the erect conidiophores but remained ultimately forming an almost spherical head. The decumbent sterile hyphae were not always distinguishable as they apparently grew through, or very close to, the soil particles or additions.

Cladosporium Link 1816 (12)

Hyphae creeping, septate, on the surface or in the substrate. Conidiophores almost erect, branched, and floccose, often forming a turf, olive colored. Conidia globose and ovoid, at first one-celled, then usually with

a cross-wall, usually greenish, terminal and then pressed to the side.

Cladosporium epiphyllum Persoon (12)

Culture Form. Colonies greenish-black, large, thick; conidiophores at first erect, then falling, pale green; conidia very numerous, soon falling from the chain, at first one-celled, then 2 to more celled, olive green, 10-22μ long x 4-6μ thick. Conidia may be 1- or 2-celled, 10-14μ x 3.8-5.2μ.

Soil Form. The fruiting heads of greenish conidia borne on dark colored erect conidiophores were very distinct. This organism was placed in Cladosporium instead of Hormodendrum as some two-celled spores were found.

Cunninghamella Thaxter 1903 (12)

Mycelium white, floccose, slightly thickened, 3-6 , continuous when young, later becoming septate, septa disposed here and there without order. Rhizoids very tenuous. Conidiophores straight, branched. The main axis, as well as the side branches, little or not septate, terminating in spherical heads, furnished with small swellings which are the points of insertion for the conidia. Conidia spherical or oval, often with an irregular outline, the external membrane spiny with needle crystals. Chlamydospores globose, intercalary in the mycelium.

Culture Form. This organism was transferred when too young and the spores did not germinate.

Soil Form. This genus was easily recognized by the erect branched conidiophores, with their slightly swollen tips on which the conidia were inserted, forming the spherical heads. The soil form did not seem to differ from the descriptions of the cultural forms.

Fusarium Link 1809 (12)

Conidial layer cushion shaped or somewhat extended without a definite limit. Conidiophores branched. Conidia terminal, single, spindle or sickle shaped, many celled with indistinct cross-walls.

Fusarium Sp. No. 1

Culture Form. All the Fusarium appeared to be of the same type. On culture the fungus was somewhat floccose, white and forming a thick, somewhat tufted surface. On Czapek's agar the reverse was white to old rose.

Soil Form. The soil form generally showed an abundant growth of white floccose mycelium with the conidiophores branching from the mycelium, terminating in heads of spores. This genus was positively identified only by spore examination with high power.

Helicoon Morgan 1893 (18)

Mycelium, mostly inconspicuous. Conidiophores short, almost lacking. Conidia filiform to screw-shaped or spiral-like. Body coiled, septate, hyaline or brightly colored.

Culture Form. Repeated attempts to isolate this organism failed.

Soil Form. The mycelium did not form a dense mass but a somewhat loose mass of hyphal strands on the surface of the substratum. The screw-shaped heads of conidia were fairly easy to observe. Most of the colonies encountered appeared on soil particles containing considerable amounts of decomposed organic matter, though some were observed on residues of the additions.

Monilia (Persoon) Saccardo (12)

Mycelium creeping, septate; conidiophores ascending or erect with dichotomous, racemose, or irregular branching. Which is sparse or abundant;

simple or branched conidial chains borne on the points of the branches or on small, blunt projections near the point. Conidia ovoid to elongate, ovoid, seldom globose, hyaline or light colored, often united by isthmus-like connecting cells.

Monilia acremonium Delacroix (12)

Culture Forms. Colonies spreading, somewhat floccose, white. Sterile hyphae creeping, hyaline, sparsely septate, with oil drops present, 4 to 5μ thick. Conidiophores erect, often united in bundles, with numerous septa, bearing the conidial chains terminally. Conidia ovate-pyriform, somewhat truncate at the base, united by small connecting cells, 12 to 15μ by 8.5 to 10μ, hyaline.

Soil Form. The fungus developed almost exclusively on the additions forming numerous thick bundles of conidiophores which completely covered the substrate in an almost solid mat, differing little in appearance from the culture forms.

Myrothecium Tode 1790 (12)

Conidial layer, shield or cushion-shaped, black, surrounded at the edge of fine hyaline cilia. Conidiophores short rod-shaped. Conidia very small, ovoid or cylindric.

Myrothecium roridum Tode (12)

Culture Form. Sporodochium shield-shaped, then confluent and sessile, black, with a white rim, 2-6 mm. in diameter. Conidiophores unbranched or forked, bush-like, 30-40μ long, 2μ wide. Conidia cylindric truncated at both ends, with 2-oil drops, smoky olive-green, 8-10 seldon 14μ long, by 2μ thick.

Soil Form. The sporodochia were circular to round with all intermediate variations. The center of a sporodochium was black to blackish-green, sessile and with a white rim of short mycelial-like filaments.

Penicillium Link 1809 (12)

Vegetative hyphae creeping, septate, branched; conidiophores erect, usually unbranched, septate, at the apex with a verticil of erect primary branches, each with a verticil of secondary and sometimes tertiary branchlets; or with a verticil of conidia-bearing cells (phialides) borne directly on the slightly inflated apex of the conidiophores; sometimes with secondary conidiophores borne on the apex of the main conidiophore; conidia borne in chains which typically form a brush-like head, not enclosed in slime; well-differentiated foot-cells not present; conidia globose, ovoid, or elliptical, smooth or rough.

Penicillium No. 1

Culture Form. Colony slow growing, white and velvety with faint almost indistinct yellow areas. Reverse practically colorless. Conidia spherical, or nearly so, averaging about 3.3μ diameter; phialides about 10 x 1.7μ; metulae 13.4 x 2.5μ and conidiophores about 3.3μ. Metulae not enlarged at apex.

Soil Form. The branched creeping vegetative hyphae on additions and the scattered white fruiting bodies on somewhat long conidiophores were distinct. Apart from the recognition of the genus by the typical brush-like appearance of the fruiting head nothing could be done toward its identification as to species in situ.

Rhizopus Ehrenberg 1820 (12)

Mycelium of two kinds, one submerged in the substratum and the other aerial, constituting the arching filaments or stolons. These stolons present from place to place the nodes on which occur the rhizoilds, which are implanted in the substratum. At these points the sporangiophores arise. They may be single but usually occur in groups of 2, 3 or more. The summit of the sporangiophore is enlarged into an apophysis, of the kind that has the columella inserted above the point where the spherical bend attaches into the filament. The sporangia, white at first, become bluish-black at maturity. They are all the same size, spherical or almost spherical, flattened at the base. Wall not cuticularized, uniformly incrusted and entirely diffluent, without leaving a basal collarette. Columella broadly subjacent, hemispherical, forming after dehiscence, by collapse, an organ of the shape of the pileus of a basidiomycete. Spore round or oval, angular, colorless or colored bluish or brown, with a cuticularized wall, smooth or striate, rarely spinulose. Zygospores naked, formed in the substratum and on the stolons. Suspensors straight, very large and swollen, without appendages.

Rhizopus nigricans Ehrenberg (12)

Culture Form. Stolons creeping, recurving to the substrate in the form of arachnoid hyphae, which are strongly raised and distant from the substrate and implanted at each node by means of rhizoids. The internodes often attain a length of 1-3 cm. and the hyphae are more or less branched. Sporangiophores rarely single, united in groups of 3-5 or more, 0.5-4 mm. in height by 24-42μ in diameter. Apophyses broad, cuneiform. Sporangia hemispheric 100-350μ. Columellae broad, hemispheric, depressed, 70μ in diameter, by

90μ in height (max. 250-320μ). Spores unequal, irregular round or oval, angular, striate, 9-12μ long by 7.5-8μ in diameter, of a grey blue. Zygospores round, or oval, 160-220μ in diameter. Exospore brown black, verrucose. Suspensors swollen, usually unequal. Azygospores present. No chlamydospores.

Soil Form. The sterile hyphae of this organism were among the first appearing in the Kubiena cultures. The stolons extended over the soil surface or grew between the soil particles and from their nodes produced one or occasionally two sporangiophores. Most of the sporangiophores had the appearance of arising directly from soil crumbs or not infrequently also from plant residues, the stolons at this stage being quite inconspicuous.

Trichoderma (Persoon) Harz 1871 (12)

Sterile hyphae creeping, septate, forming a flat, firm turf. Conidiophores erect, arising from short, branched side-branches, branching usually opposite, not swollen at the apex and bearing terminally the conidial heads. Conidia small, mostly globose, bright colored or hyaline.

Trichoderma lignorum (Tode) Harz (12)

Culture Form. Colonies on Czapek's agar broadly spreading, hyaline; fruiting areas appear as tufts, white at first, and becoming various deep green shades with age; reverse colorless. Conidiophores arise as branches of aerial mycelium septate, up to 70μ in height by 3.0μ in diameter, di- or tri-chotomously branched, occasionally forming whorls. Conidial heads up to 10μ in diameter; conidia globose to ovoid, smooth, 3.8 to 3.2μ in diameter.

Soil Form. The soil form appeared as small tufted areas. The upper surface of the tufts were light green with white mycelium below.

Trichothecium Link 1824 (12)

Hyphae creeping. Conidiophores erect, septate unbranched. Conidia terminal, single, 2-celled, hyaline or bright colored.

Trichothecium roseum Link (12)

Culture Form. Turf forming a powdery case, widespread, mold-like or arachnoid, white, finally pink, formed of creeping, branched, septate white hyphae. Conidiophores erect, little or non-septate, usually unbranched and scarcely swollen at the tip. Conidia acrogenous, single, one after another, but remaining attached and forming a head by apical growth, pear-shaped, 2-celled, the apical cell being larger, hyaline than pink, 12-18μ long by 8-10μ broad.

Soil Form. The conidial heads were borne on simple erect conidiospores arising either from hyphae decumbent on the substrate or sometimes from hyphae extending across soil spaces and usually consisted of 5 to 6 spores, though occasionally one only was present. The conidial heads in incident light sparkled like crystals.

Volutella Tode 1790 (12)

Fruit layer superficial, disc-shaped or somewhat globose, sessile or on a short stalk, regularly formed, with long bristles or spines at the margin and sometimes in the middle of the disc. Conidiophores thickly gregarious, covering the entire disc, at the base united with the spines as branches, usually several times branched, the last branches forming a thick hymenium of fine, sterigma-like stalks. Conidia terminal, formed in masses small, ovoid or elliptic[illegible] hyaline.

Culture Form. This organism was not obtained in culture.

Soil Form. The sporodochia were large, erumpent, somewhat spherical, dark yellow in color with simple filaments around the sporodochia. Abortive branching was sometimes observed.

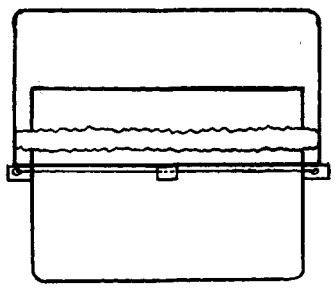


Fig. 1. Culture dish for Kubiëna technique

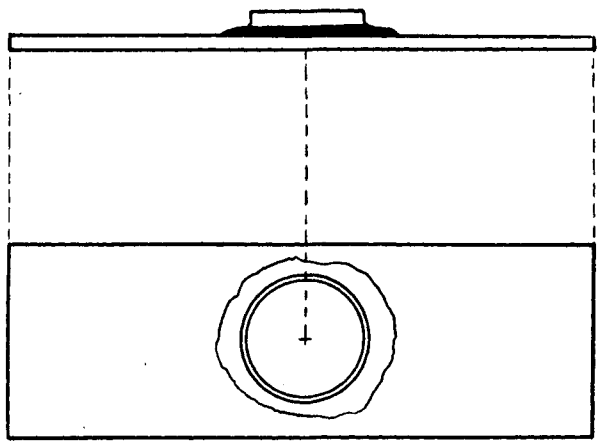


Fig. 2. Modified well slide for "bodenstaub" technique

Plate 1

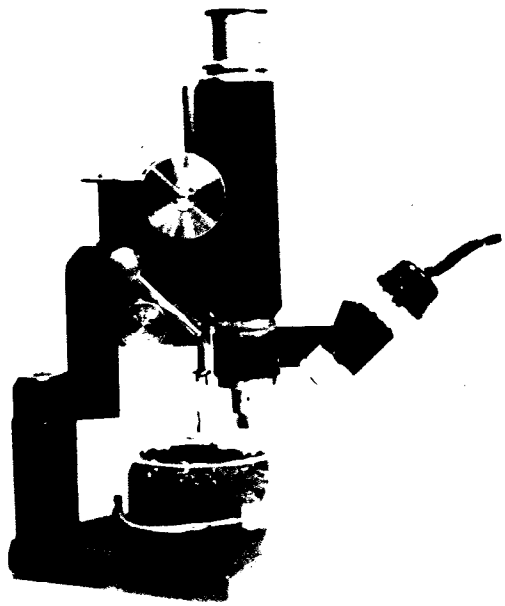


Plate 2

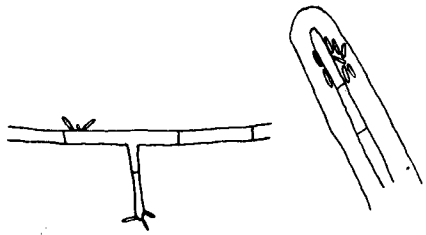


Fig. 1. Bacteria in water film around mycelium

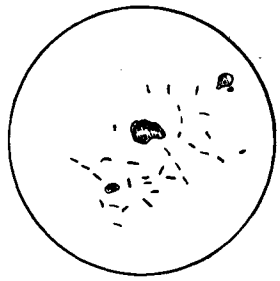


Fig. 2. Bacteria surrounding soil particles

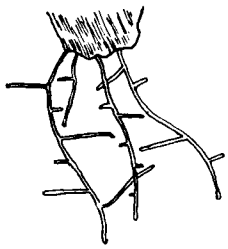


Fig. 3. Actinomycetes developing from soil particle.

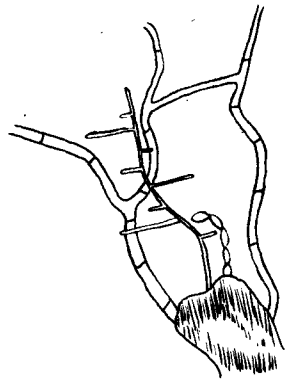


Fig. 4. Actinomycetes, fungous hyphae and spores

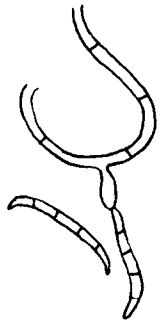


Fig. 5. Fusarium fruiting

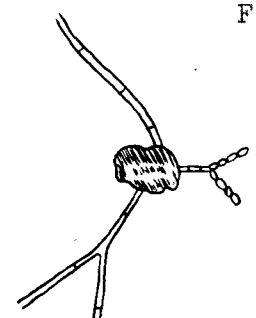


Fig. 6. Oospora fruiting

Fig. 7. Bacteria in water film around soil particle

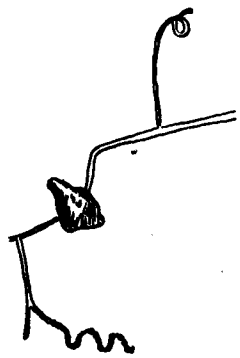


Fig. 8. Sporulating actinomycetes

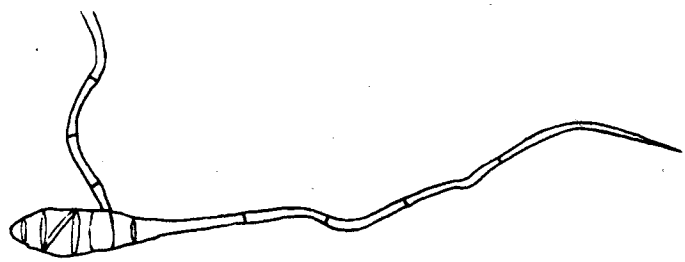


Fig. 9. Germinating Alternaria spore

Plate 3

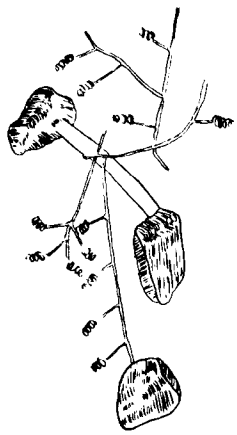


Fig. 1. Helicoon colony from soybean addition

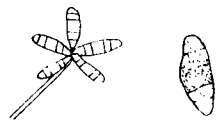


Fig. 2. Acrothecium fruiting head and spore

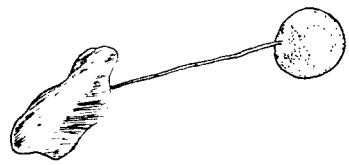


Fig. 3. Rhizopus developing from soil particle

Fig. 4. Penicillium fruiting from soil particle

Fig. 5. Trichothecium fruiting head and spore

Plate 4

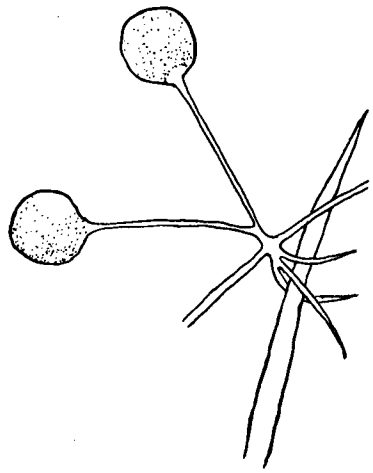


Fig. 1. Rhizopus attached to soybean leaf hair

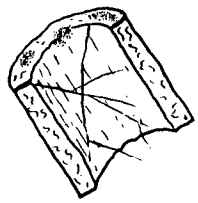


Fig. 2. Actinomycetes colonies and fungous filaments in soybean stem

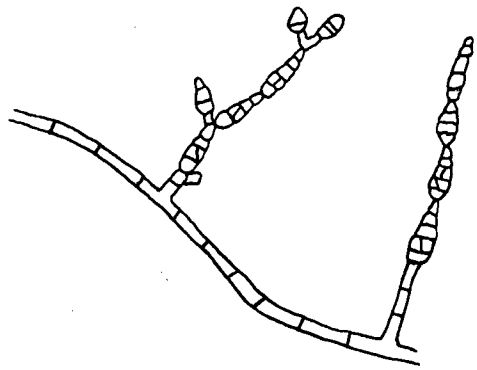
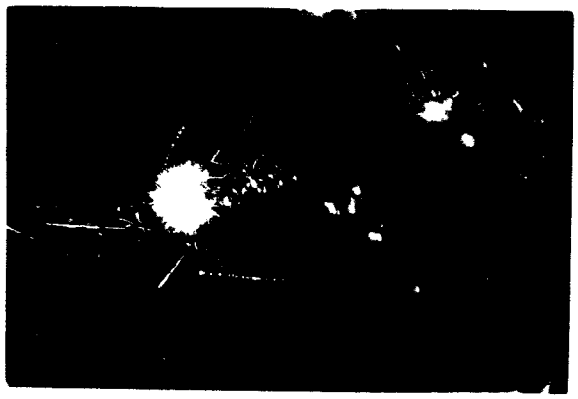


Fig. 3. Alternaria mycelium and spores

Plate 5

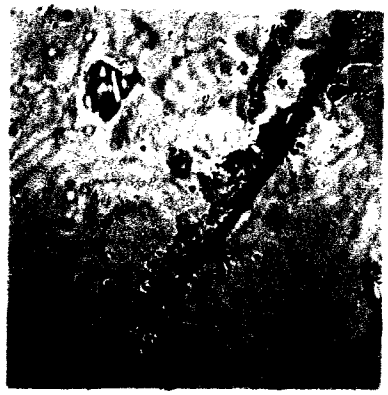


Colony of actinomycetes developing on a piece of alfalfa leaf in Tama silt loam. About 35X.

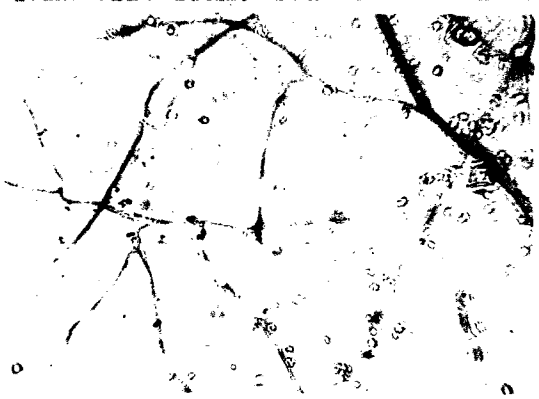
Mycelium extending from sweet clover stem to soil particles. About 200X.

Sporangium of Rhizopus nigricans developing from soil particle in Tama silt loam. About 35X.

Alternaria spores developing from alfalfa leaf in Tama silt loam. About 72X.



Oospora hypha. (From buried slide in contact with alfalfa in Tama silt loam. About 800X.



Mycelium of actinomycetes. (From buried slide in contact with alfalfa in Tama silt loam. About 800X.

Bacteria near mycelium. (From buried slide in contact with alfalfa in Tama silt loam. About 800X.

Plate 6

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